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Report on Algal Toxicity Tests on Selected Office of Toxic Substances (OTS) Chemicals

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REPORT ON ALGAL TOXICITY TESTS ON SELECTED OFFICE OF TOXIC SUBSTANCES (OTS) CHEMICALS

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24 FEBRUARY 1989

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Algal (Selenastrum capricornutum) assays were performed on 11 chemicals selected from a larger list of candidate chemicals provided by the EPA's Office of Toxic Substances (OTS). Limited chemical analysis of those chemicals that could be tested readily at ERL-C was performed in support of the bioassays. Of the 11 chemicals tested, two (tetraethyl ammonium chloride and butyl mercaptan) were judged non-toxic to the algae by virtue of the calculated EC_{50} exceeding 1000 mg/L. Three chemicals were extremely toxic (EC_{50} < 10 mg/L; methyl vinyl ketone, octylamine, and phthalic anhydride); three were highly toxic (EC_{50}> 10mg/L but <100 mg/L; azosulfamide, methyl acrylate, and nitrobenzene); and three were moderately toxic (EC_{50}>100 mg/L but <1000 mg/L; glyoxal, sodium dodecylbenzenesulfonate, and glycidol). This work contributes to the toxicity data base assembled on these chemicals. Previously, algal toxicity information was lacking for these compounds.

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EXECUTIVE SUMMARY

Algal (Selenastrum capricornutum) assays were performed on 11 chemicals selected from a larger list of candidate chemicals provided by the EPA's Office of Toxic Substances (OTS). Limited chemical analysis of those chemicals that could be tested readily at ERL-C was performed in support of the bioassays. Of the 11 chemicals tested, two (tetraethyl ammonium chloride and butyl mercaptan) were judged non-toxic to the algae by virtue of the calculated EC_{50} exceeding 1000 mg/L. Three chemicals were extremely toxic (EC_{50} < 10 mg/L; methyl vinyl ketone, octylamine, and phthalic anhydride); three were highly toxic (EC_{50} > 10 mg/L but < 100 mg/L; azosulfamide, methyl acrylate, and nitrobenzene); and three were moderately toxic (EC_{50} > 100 mg/L but < 1000 mg/L; glyoxal, sodium dodecylbenzenesulfonate, and glycidol). This work contributes to the toxicity data base assembled on these chemicals. Previously, algal toxicity information was lacking for these compounds.

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ATTENTION

AS NOTED IN THE NTIS ANNOUNCEMENT, PORTIONS OF THIS REPORT ARE NOT LEGIBLE. HOWEVER, IT IS THE BEST REPRODUCTION AVAILABLE FROM THE COPY SENT TO NTIS.

I. Introduction

This report presents the results of the algal toxicity tests performed on 11 chemicals selected from a list designated by EPA's Office of Toxic Substances (OTS). Important procedural modifications were necessary to complete the tests of these substances. This report describes the biological, chemical and statistical methods used to complete the analyses. Interpretations of toxicity are provided.

II. Chemicals Analyzed

The following list of chemicals was selected from a master list provided by OTS. Feasibility of testing the chemical in an aqueous matrix and limiting health and safety concerns were the primary criteria for selection. To optimize personnel safety, the least toxic chemicals were favored. Testing was performed between April and September 1989.

glyoxal
sodium dodecylbenzenesulfonate
tetraethyl ammonium chloride
phthalic anhydride
azosulfamide
glycidol
octylamine
nitrobenzene
methyl vinyl ketone
methyl acrylate
butyl mercaptan

Two other chemicals were candidates for testing. Methyl methacrylate was not approved for testing by the Environmental Research Laboratory - Corvallis (ERL-C) Health and Safety Committee, "due to its possible explosive polymerization." Dibutyl phosphate was not available for testing.

Methyl vinyl ketone, methyl acrylate, and butyl mercaptan, due to their low vapor pressures and possible carcinogenic properties, had to be tested in a ventilated exposure chamber. This special requirement necessitated building an appropriate testing chamber and delayed testing until late August 1989.

III. Methods

The test protocol requested by OTS was the standard procedure from the Federal Register (Vol. 50; No. 188; Part 797; Sec. 797.1050, Algal Acute Toxicity Test). Since that publication, several substantive methods modifications have been promulgated in EPA publications (Greene, et al., 1988; Weber, et al., 1989). Attached in Appendix A is a copy of the ERL-C standard operating procedure for the algal assay test. This procedure with the specified modifications requested by OTS (also in Appendix A) was followed in testing these materials. Variances from procedures in these documents included absence of subculturing, limitations in chemical analyses (see Chemical Analysis section), and minimum control yields and use of chelating agents, which are discussed below. Although tests were counted every day, the final EC_{50} value was determined using only the 96 hour counts. Counts for each day are reported as "raw data," in Appendix C. The records of the daily monitoring of the light intensity and shaker speed for each test are also presented in Appendix C.

All test compounds were subjected to range-finding tests. If the EC_{50} was greater than 1000 mg/L in the range-finding test, no definitive test was run.

A. Modifications to Federal Register Procedure

1. Chelating Agents and Minimum Cell Counts at 96 hours.

The procedure published in the Federal Register stipulates: "Algal growth in controls should reach the logarithmic growth phase by 96 hours at which time the number of algal cells should be approximately ... 3.5 x 10^6/ ml for Selenastrum. If growth in controls does not reach this logarithmic phase within this 96 hour period, the test is invalidated and should be repeated." The procedure also states: "No chelating agents should be included in the nutrient medium used for test solution preparation." The EPA publications cited above define media with EDTA added as a chelating agent. Recent ASTM proceedings concur that EDTA is required to support sufficient algal growth. The ERL-C standard Algal Assay Media contains 300 ug/L EDTA, and the growth in controls does reach the log phase, although the number of algal cells at 96 hours averages somewhat less than what is suggested in the Federal Register. The proposed New Standard Guide for Conducting Static Toxicity Tests With Microalgae (ASTM E1218) requires that for a test to be valid, 10^5 cells/ml must be present at 96 hours.

As verification of the EDTA requirement, negative control tests in media without EDTA failed to achieve the ASTM minimum growth criterion (See results in Appendix D).

B. pH Adjustment

In order to test Glyoxal, pH adjustment was necessary. The analysis of this compound was more extensive than was originally anticipated because of the tendency of the Glyoxal to cause a drop in pH below the tolerable range of the test organism during the course of the test.

During the first range finding test the pH of the 100 mg/L concentration (the second highest) was above the lower limit of 6.0 for the green algae Selenastrum capricornutum. However, the pH of the 1000 mg/L concentration was below that limit (about 4.5). Because the EC_{50} for that test fell between 100 and 1000 mg/L, it was impossible to distinguish between pH caused toxicity and other toxic effects. Accordingly, in compliance with ERL-C QA requirements, the pH was adjusted in subsequent tests.

An initial adjustment to pH 7.5 failed to keep the pH within the tolerance range for the duration of the test. Two more tests were run in which the pH was adjusted over a longer period of time and to a higher pH (9.5) to possibly "saturate" the buffering capacity of the solution or to offset the drop below 6 until after the 96 hour exposure. We were hesitant to go ahead and run the definitive test between 100 and 1000 mg/L not knowing at what point the pH would fall below the lower limit and not knowing whether a stabilized pH could be attained.

After the third attempt at stabilizing an adjusted pH above the lower limit at the 1000 mg/L concentration with no success, we decided to run the definitive test on an adjusted sample, fully expecting that during testing some of the concentrations would fall below the pH range of the algae. After testing, the pH of the concentrations used in the determination of the EC_{50} value were all within the acceptable range for the algae (the data from concentrations that were out of the acceptable range were not used in the EC_{50} determination), thus the pH had no influence on the EC_{50} value. Removing these data points was possible because the full response range (no effect through complete inhibition) occurred at concentrations that were within the pH range of the algae. Since the sample was originally adjusted, however, the adjustment solution may have influenced the EC_{50} value.

No other compound required pH adjustment for a definitive test. The highest two concentrations in the range-finding test of phthalic anhydride were pH adjusted but because the toxicity for that test fell below the adjusted concentrations no adjustment was necessary for the definitive test

C. Derivation of Algal Assay Results

1. Data Reduction

One ml samples were pipetted from the test flasks and counted on an electronic particle counter (Coulter Counter, Model ZM). Counts were multiplied by the appropriate dilution factor. These counts, cells/ml, were then multiplied by the mean cell volume (MCV), and a conversion factor (3.6×10^{-7}) to get the mg/liter dry weight. If a cell count for a negative control flask was 500 or greater (minus the background) at a dilution factor of 200, the flask contained $\geq 10^{5}$ cells/ml and was valid according to the proposed New Standard Guide for Conducting Static Toxicity Tests With Microalgae (ASTM E1218). The mg/L dry weight was calculated for each flask and compared to the average mg/L dry weight of the three control flasks after the inoculum had been subtracted in the formula t/c, where t was the test concentration yield in mg/L dry weight and c was the control. This number was multiplied by 100 to get the percent growth as compared to the control.

For example, in a test with an inoculum mg/L dry weight of 0.16, a flask had a yield of 5.40 mg/L dry weight (minus inoculum = 5.24) which was divided by the average control yield of 32.23 mg/L dry weight (minus inoculum = 32.07) to get 5.24/32.07 = 0.1634; this number was multiplied by 100 to get 16.34 percent of control yield.

2. Statistical Analysis

After the cells per milliliter, milligrams per liter dry weight, and percent effect were calculated using a Lotus spreadsheet program, the median effect values were derived from a regression analysis using the Statgraphics program (a commercial statistical software package). The following is a brief explanation of the methods used, and the reasoning those methods, for determining toxicity test results.

Options for statistical analysis are many, and certainly other approaches to data reduction could generate alternative interpretations of the data.

The test concentration yields, expressed as a percent of the control, were plotted against the toxicant concentrations in the Statgraphics program. Regressions were performed using linear, multiplicative, and exponential models on the percent of control yield data. The model that had the highest r-squared value (goodness of fit) was chosen from the options, and the EC_{50} was calculated from that model.

Fiducial limits[1] (commonly mislabeled confidence limits) were obtained mathematically using an in-house Lotus spreadsheet program that calculates the limits from outputs generated from the Statgraphics program.

a. Approach

As variables were entered into the Statgraphics program, asymptotic portions were removed. Removal of these asymptotic portions facilitated statistical analysis. ERL-C standard procedure dictates removal of data sets that were either redundant because they were equivalent to the control yield or showed complete inhibition (all dead). All points associated with a particular toxicant concentration were omitted when data was truncated. All concentrations above or below an omitted set were also omitted.

For example, if all concentrations up to and including 25 mg/L had growth equivalent to the control, data for those concentrations below 25 mg/L would be omitted. Similarly, if concentrations of 500 mg/L and over showed complete effect, all those greater than 500 mg/L would be omitted.

What constitutes "equivalent to the control" or "complete effect" is often a subjective decision. Data truncation can profoundly influence the estimated median effect. For this reason we present all the raw data, in order to facilitate any recalculation desired.

To minimize interpretive decisions, it was originally decided not to truncate the data at all for this project. However, due to the test design (the dilution scheme) of some tests, regression without truncation was not optimum. The concentrations in the range-finding tests were far apart relative to the "response window." This caused the effective response area to be so small, median estimates using all the points were at times anomalous.

---

[1] Fiducial limits (intervals) are the 95% (two standard deviations) limit on either side of a calculated number for the independent variable (the toxicant) which lies along the horizontal axis. These are the toxicant amounts (limits) between which (interval) there is a 95% probability that the 50% response will occur.

Confidence limits (intervals) are the 95% (two standard deviations) limit on either side of a calculated number for the dependent variable (the response) which lies along the vertical axis. These are the response (as percent of control yield) amounts (limits) between which (interval) there is a 95% probability that (at that particular calculated toxicant amount) the response will occur.

For example, an EC50 is 16.35 mg/l with 95% fiducial limits of 10 and 23 mg/l. There is a 95% probability that the calculated EC50 (50% response) of 16.35 mg/l is between 10 and 23 mg/l. The confidence limits for that EC50 of 16.35 mg/l may be 20 and 75. There is a 95% probability that at the toxicant level of 16.35 mg/l the response is between 20 and 75%.

Truncation was kept to an minimum in analyzing the data, and the amount of truncation can be determined from Table 1 by looking at the Degrees of Freedom (DOF) used in each EC_{50} calculation. The degrees of freedom can be figured from the number of data points minus one, so if an entry reads 14 DOF, that means that fifteen flasks were used in data analysis, comprised of three replicates of five different toxicant concentrations. The number of flasks actually tested is the number of concentrations multiplied by the number of replicates. If DOF + 1 is not equal to concentrations x replicates, the data was truncated. A difference of 3 would indicate one concentration (three replicates) was omitted. The actual data points used in the calculations can be seen in the graphs in Appendix B. Again, raw data used in the regression graphs is presented in Appendix C.

Three data points (one concentration, the highest one) were removed for sodium dodecylbenzenesulfonate, methyl vinyl ketone and methyl acrylate. Removing the highest concentration when there is "redundant death" is a common truncation practice. The highest concentration for glyoxal was removed because the pH at that toxicant level was below the tolerance range for the organism. All other data points in the definitive tests were used.

IV. Chemical Analysis

ERL-C had no previous experience analyzing the specific organic compounds tested in this project. The analyses we completed in-house were on the original sample concentrations before testing and on the final filtered solutions for each concentration. Some sample levels were verifiable using gas or liquid chromatography, others required verification by some other method.

The Chemical Analyses Summary (Table 2) presents the results for the analyses completed. Analyses not performed include octylamine and methyl vinyl ketone (the definitive test amounts were below the analytical detection limit for our instruments), sodium dodecylbenzenesulfonate and tetra ethyl ammonium chloride (a derivatization procedure to create an analyzable compound would be required), glyoxal (a standard simply was not detectable with any of our available instrumentation), and butyl mercaptan (samples were mistakenly disposed of before chemical analysis could commence).

At present the unanalyzed test solutions (except for butyl mercaptan), both from before and after testing, are being held in refrigerated storage at 4 degrees C. Additional resources are needed to design methods to test these materials. Alternatively, samples could be forwarded to a designated laboratory for analysis.

V. Results

The EC_{50}'s, fiducial limits, and selected test parameters associated with the tests performed are presented in Table 1. Results from both the range-finding and definitive tests are included in the table for those materials on which both tests were run. Each entry in the table has an accompanying analysis of variance (ANOVA) table and regression graph, in Appendix B, that was used to generate the calculated median effect value. The raw data used to generate the graphs in Appendix B, including daily counts for the range-finding tests, are in Appendix C.

Of the 11 chemicals tested, two (tetraethyl ammonium chloride and butyl mercaptan) were judged to be relatively non-toxic to the algae by virtue of the calculated EC_{50} exceeding 1000 mg/L. Three chemicals were extremely toxic ($EC_{50} < 10$ mg/L; methyl vinyl ketone, octylamine, and phthalic anhydride); three were highly toxic ($EC_{50} > 10$ mg/L but < 100 mg/L; azosulfamide, methyl acrylate, and nitrobenzene); and three were moderately toxic ($EC_{50} > 100$ mg/L but < 1000 mg/L; glyoxal, sodium dodecylbenzenesulfonate, and glycidol).

VI. Discussion

Tetra ethyl ammonium chloride and butyl mercaptan did show toxicity in range-finding tests at the highest concentration, but the inhibition was in both cases less than 50% so no definitive tests were run. An EC_{50} of 1068 mg/L for butyl mercaptan and an EC_{50} of 1406 mg/L for tetra ethyl ammonium chloride are offered on the Algal Assay Summary (Table 1); but these calculated median effect estimates are extrapolations beyond the highest concentration, are from single replicate tests, and have no fiducial intervals associated with them. Because of this ambiguity, the toxicity for these chemicals should be most properly described as "greater than 1000 mg/L" (>1000 mg/L). Since toxicity was observed, however; and since the EC_{50} value could fall quite close to 1000 mg/L if tested with a more robust test design, the information was included.

The first entry for the range-finding test for phthalic anhydride is quite dissimilar to the definitive test entry due to the fact that the two highest concentrations (1000 and 100 mg/L) in the range-finding test were pH adjusted while the lower four were not.

These two high concentrations were the only ones out of the organism's pH range. When all the data points were examined, two distinct slopes could be seen. A toxic response was evident in the lower unadjusted group (the second entry for the range-finding test for that material), and the design of the definitive test was based on that data. None of the range-finding test results should be heavily relied upon due to the single replicate test design; in all cases the results of a definitive test should be reported instead of those from a range-finding test. The results of the range-finding tests are included only to provide the data base on which decisions about concentrations for the definitive test were made, and to illustrate the correlation between the range-finding and definitive tests.

All definitive test median effect estimates have fiducial limits associated with them except for azosulfamide. The median effect estimate for that compound had no upper fiducial limit due in part to the fact that the EC_{50} fell relatively close to the highest concentration (Statgraphics will not extrapolate a fiducial limit beyond the highest concentration); and in part because the second highest concentration was not generally more toxic than the third. The data look relatively sound (see ANOVA table and regression graph in Appendix B), and an upper fiducial limit might be obtained using another procedure.

REFERENCES

Federal Register (Vol. 50; No. 188; Part 797; Sec. 797.1050, Algal Acute Toxicity Test).

Greene, et al., Protocols for Short Term Toxicity Screening of Hazardous Waste Sites. EPA/600/3-88/029. February, 1989.

Weber, et al., Short-Term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms, Second Edition. EPA/600/4-89/001. March, 1989.

Standard Operating Procedures for ERL-C Bioassays. Site Assessment Team, Environmental Research Laboratory - Corvallis, USEPA, 200 SW 35th St., Corvallis, OR 97330. 1989, unpublished.

New Standard Guide for Conducting Static Toxicity Tests With Microalgae (proposed). ASTM E1218.

Statgraphics is a registered trademark of Statistical Graphics Corporation.

ALGAL ASSAY RESULTS SUMMARY: CHEMICALS TESTED FOR THE OFFICE OF TOXIC SUBSTANCES (OTS) Table 1

DATE: 06-Mar-90

CHEMICAL	CHEMISTRY NUMBER	TEST DATE	TEST TYPE	EC50 (mg/L)	LFL= (mg/L)	UFL= (mg/L)	R-SQUARED (R^2)	Number of CONCS.	Number of REPS.	HIGH CONC. (mg/L)	LOW CONC. (mg/L)	Degrees of Freedom
Glyoxal	OTP14600	04-03-89	D	148.96	0.00	348.59	82.29	5	3	1000	62.50	$11
	OTN09600	02-28-89	R	66.48	0.00	551.45	88.67	6	1	1000	0.01	5
Sodium	OTN18600	05-08-89	D	70.27	45.11	95.42	94.72	5	3	250	15.63	$11
dodecylbenzenesulfonate	OTN18600	05-01-89	R	171.96	55.93	287.99	99.26	6	1	1000	0.01	5
Tetraethyl ammonium chloride	OTN20600	05-15-89	R	1405.9+	23.04	2788.76	71.05	6	1	1000	0.01	5
Phthallic anhydride	OTN23600	06-05-89	D	4.14	0.00	10.91	82.97	7	3	20	0.31	20
	OTN21600	05-23-89	R	78.53	0.00	1353.33	53.03	6	1	1000	0.01	5
				0.147##	0.06	0.35	78.73	4	1	10	0.01	3
Azosulfamide	OTN25600	06-26-89	D	28.93	4.81	174.05	71.41	6	3	100	3.12	17
	OTN25600	06-19-89	R	32.77	0.00	70.53	95.64	6	1	1000	0.01	$4
Glycidol	OTN25601	06-26-89	D	144.70	52.20	237.20	95.15	5	3	500	31.25	14
	OTN25601	06-19-89	R	53.31	0.00	167.88	99.29	6	1	1000	0.01	5
Octylamine	OTN25602	06-26-89	D	0.22	0.03	0.42	94.40	6	3	1	0.03	17
	OTN25602	06-19-89	R	0.07	0.00	1.28	99.00	6	1	1000	0.01	$2
Nitrobenzene	OTN29600	07-24-89	D	23.78	8.58	38.98	96.69	5	3	100	6.25	14
	OTN29600	07-17-89	R	20.79	3.45	38.13	99.04	6	1	1000	0.01	$4
Methyl vinyl ketone	OTN34800	08-28-89	D	0.13	0.05	0.21	88.98	5	3	0.5	0.03	$11
	OTN34800	08-21-89	R	0.12	0.00	0.61	99.83	6	1	1000	0.01	$2
Methyl acrylate	OTN37801	09-18-89	D	18.57	3.74	33.41	94.88	6	3	100	3.13	$14
	OTN37801	09-12-89	R	15.53	0.00	46.78	96.03	6	1	1000	0.01	$4
Butyl mercaptan	OTN37800	09-12-89	R	1068.3+	0.00	5478.24	92.44	6	1	1000	0.01	5

D	Definitive
R	Range-Finding
LFL	Lower Fiducial Limit
UFL	Upper Fiducial Limit
$	Data was truncated (see text).
=	Fiducial Limits obtained mathematically.
##	Subset of range-finding test (see text).
+	EC50 Above high concentration (see text).

CHEMICAL RESULTS SUMMARY: CHEMICALS TESTED FOR THE OFFICE OF TOXIC SUBSTANCES (OTS) DATE: Feb-90 TABLE 2

CHEMICAL	CHEMISTRY NUMBER	TEST DATE	TEST TYPE‡	NUMBER of CONCENTRATIONS	NOMINAL CONCENTRATION (MG/L)	ANALYSIS PRIOR TO TESTING (MG/L)			ANALYSIS AFTER TESTING (MG/L)			ANALYTICAL METHOD
						REP 1	REP 2	AVG.	REP 1	REP 2	AVG.	
Phthalic anhydride	OTN23600	06-05-89	D	7	20	20.5	20.9	20.7	NA	NA	0.0	HPLC#
					10	0.4	BD	0.2	6.9	7.3	7.1	
					5	0.3	0.2	0.2	4.1	4.1	4.1	
					2.5	2.6	2.2	2.4	2.0	1.8	1.9	
					1.25	1.2	1.1	1.1	0.3	0.4	0.3	
					0.625	0.5	1.0	0.7	0.5	1.0	0.8	
					0.3125	0.1	0.4	0.3	0.2	0.6	0.4	
Azosulfamide	OTN25600	06-26-89	D	6	100	95.0	95.0	95.0	98.0	98.0	98.0	UV-VIS@
					50	49.3	49.3	49.3	50.7	50.6	50.7	
					25	25.0	24.9	25.0	25.9	25.8	25.9	
					12.5	12.2	12.3	12.3	12.9	12.9	12.9	
					6.25	6.4	6.4	6.4	14.2	14.0	14.1	
					3.125	3.2	3.2	3.2	3.8	3.8	3.8	
Glycidol	OTN25601	06-26-89	D	5	500	198.1	195.1	196.6	NA	NA	0.0	HPLC#
					250	BD	BD	0.0	BD	BD	0.0	
					125	BD	BD	0.0	BD	BD	0.0	
					62.5	BD	BD	0.0	BD	BD	0.0	
					31.25	BD	BD	0.0	BD	BD	0.0	
Nitrobenzene	OTN29600	07-24-89	D	5	100	84.0	82.6	83.3	27.3	26.7	27.0	HPLC#
					50	42.3	42.3	42.3	12.3	12.2	12.3	
					25	21.2	20.9	21.1	5.2	5.2	5.2	
					12.5	10.6	10.5	10.5	2.6	2.5	2.5	
					6.25	5.0	4.9	4.9	1.0	1.1	1.1	
Methyl acrylate	OTN37801	09-18-89	D	6	100	67.7	68.3	68.0	BD	BD	0.0	HPLC#
					50	42.6	40.6	41.6	BD	BD	0.0	
					25	21.3	21.4	21.4	BD	BD	0.0	
					12.5	10.3	10.2	10.2	BD	BD	0.0	
					6.25	6.0	6.1	6.1	BD	BD	0.0	
					3.125	3.1	3.2	3.2	BD	BD	0.0	
Butyl mercaptan	OTN37800	9-12-89	R	6	1000	541.0	331.0	436.0		Not Available		HPLC#
					100	BD	BD	0.0				
					10	BD	BD	0.0				
					1	BD	BD	0.0				
					0.1	BD	BD	0.0				
					0.01	BD	BD	0.0				

‡ R = Range-finding; D = Definitive.

High Performance Liquid Chromatograph

@ Ultraviolet Visible Spectroscopy

BD Below Detection Limit. The final concentrations for methyl acrylate and all but the highest concentration for glycidol and butyl mercaptan could not be detected.

NA Not available.

APPENDIX A

ERL-C STANDARD OPERATING PROCEDURE FOR THE ALGAL ASSAY

Hazardous Waste Assessment Team
NSI-ES
ERL-C

Date : January 30, 1989

To: Distribution

From: Michael Bollman

Subject: Test Procedure for OTS Chemicals

After careful examination of the correspondence between our lab and the Office of Toxic Substances (OTS) it has become evident that OTS would like us to follow procedures that differ markedly from our standard operating procedures. This memo is to outline what procedures we will follow for bioassays of all the OTS chemicals which we test.

1. An algal range finding test is required on a logrithmic scale from the saturation point of the compound in water down to the detection level (e.g. 1000, 100, 10, 1.0, .1, .01 mg/Liter). At least 5 concentrations must be run. This test will be run using only one replicate.

2. All testing must be done in glassware pre-conditioned with a solution of the appropriate test concentration. To accomplish this and to provide enough solution for possible chemical analysis at a later date (see #3 below), our procedure will be to mix up 300 mls of each test concentration in a 500 ml flask (exactly the same procedure we do now but 6 times as much) the day before testing is to go on (or the Friday before), and then on the day of testing 50 mls of each concentration is buretted into one of the three replicate flasks for each concentration, then rinsed through the other two, and then discarded. Each replicate flask is then filled with 50 mls of the proper concentration, and the remaining solution from the 500 ml flask is poured into a 100 ml Nalgene bottle for storage.

3. Samples of each concentration before and after testing are to be kept in frozen storage for possible future chemical analysis. Beginning samples are obtained as above in #2, final samples are obtained by combining all three replicates (after sampling for testing on day four) and withdrawing approximately 100 mls.
In addition, after the final sample has been removed for storage, an aliquot of the remaining pooled sample is centrifuged down (or filtered) and the algal cells themselves saved in frozen storage for possible future chemical analysis.

4. If the algal range-finding test shows toxicity (EC50) below the lowest concentration tested or above the highest concentration tested, a definitive test will not be run. If an EC50 is obtained within the bounds of the range-finding test a definitive test will be run using 5 concentrations in which the ratio is 2.0 (e.g. 1, 2, 4, 8, 16 mg/liter). If a definitive test is run, the samples held from the range-finding test for chemical analysis can be discarded. If a definitive test is not run, they must be kept.

5. No chelating agents can be used in the media so we must mix up same Algal Assay Media without EDTA. This media will be used for all tests of OTS samples.

6. All stock solutions and serial dilutions must be made with media, not R.O. water.

7. Both the range-finding and definitive tests must be counted daily (i.e. 24, 48, 72, and 96 hours). In addition, light intensity and shaker oscillation must be recorded daily (this can be incorporated into the sampling procedure). Tests should be sampled at the same time each day, so a space will be made on the data sheet to record this. To facilitate ease of these daily observations, tests will be started on Monday and run through Friday. Tentatively, testing for Glyoxal will begin on Monday, February 27th.

Other parameters of testing will remain the same as usual; a 10,000 cell inoculum will be used, positive and negative controls set up in triplicate, Lotus (algae program) and Statgraphics on the results, subcultures of concentrations with counts under 1300 cells/ml at 1:20 dilution (actually the lowest concentration that completely inhibited algal growth), etc.

In calculating the results, observations of cell size and number in relation to biomass are to be included, and tests in which the control yield is less than 1.5×10^6 must be repeated.

Distribution: Kapustka
Baune
DeWhitt
Nwosu
Smith

Selenastrum capricornutum 96-Hour Static Chronic Toxicity Bioassay

Introduction: Unicellular algae have been extensively used for toxicity bioassays. Their importance in oxygen evolution and as primary producers make them suitable environmental indicator organisms. The procedure presented here is similar, with slight variations, as those presented by Porcella (1983) and, Horning and Weber (1985). An inoculum (approximately 10,000 cells/ml) of *Selenastrum capricornutum* is incubated for 96 hours with varying concentrations of one of the following, diluted with algal assay medium: surface water, ground water, or soil or sediment eluate. A negative control of algal assay medium is used as a reference for toxicity evaluations. Toxicity assessments are made by determining an EC_{50} value (the concentration causing inhibition of growth by 50 percent relative to measured growth in the control).

Room 274 - Environmental Conditions for Culture and Test Incubation

Environmental Chamber for *Selenastrum capricornutum*

Temperature: 24 +/- 2°C
Light: continuous, "cool white " fluorescent, 360-440 foot candles (= 4304 +/- 10% lux)
Shaker rate: 100 rpm +/- 10%
All references to room 274 indicate the above conditions.

Culture Methods: A sample of *Selenastrum capricornutum* culture (ATCC Culture No. 22662) (which can be obtained from the American Type Culture Collection) has been maintained at CERL by consecutive aseptic transfer to 50 mls of liquid algal assay media (see AAM). These transferred cultures are used to maintain a continuous stock culture. Daily aseptic stock transfer consists of pipetting approximately 1ml of a 3 to 7 day old stock culture, that has been incubated in room 274, into each of two sterile, 125ml Erlenmeyer flasks each containing 50mls of fresh AAM. (Newly transferred flasks are referred to as "isolates".) The grown stock culture is taken from room 274, algal culture room, to lab 250 for isolation transfer. A sterile 5ml pipet or an automatic pipet with sterile tip and Bunsen burner are used to reduce contamination during transfer. (Between 0.5 and 2.5mls can be used for daily transfer, the lower the amount transferred, the longer the time period before the culture can be used for testing purposes. The greater the amount transferred the quicker the culture reaches nutrient limitation.)

Depending on the amount transferred, newly inoculated flasks require 3 to 7 days incubation, in room 274, before growth is sufficient to be used for a test or another stock transfer.(When 1ml is used consistently for daily stock transfer, growth for use in toxicity tests or stock transfer is generally sufficient after 3 or 4 days.)

Monthly isolates from liquid culture or isolated plate colonies are plated on approximately 1% AMM agar (see AAM agar below). A flame sterilized streaking loop is used to streak algae on the plate. The plates are incubated on a shelf in room 274 until isolated colonies can be seen on the plate. A single colony is then transferred via a sterile streaking loop to 50mls of fresh liquid AAM. Once this has grown, it is used for the daily isolate. All isolate transfers, daily and monthly, are recorded in the Isolate Book and Culture Log, located in room 274.

NUTRIENT STOCK SOLUTIONS FOR ALGAL ASSAY MEDIA
Spikes (1-5) for Liquid Algal Assay Media (AAM)

Stock Spike #1 of 5

$NaNO_3$	6.375g.
$MgCl_2 \cdot 6H_2O$	3.041g.
$CaCl_2 \cdot 2H_2O$	1.103g.

Dissolve in 200 mls of RO water and bring up to 250ml volume in a volumetric flask.

Stock Spike #2 of 5

	weigh out	dissolve in RO water	pipet out
H_3BO_3	0.464g.	100 mls	10 mls
$MnCl_2 \cdot 4H_2O$	1.038g.	100 mls	10 mls
$FeCl_3 \cdot 6H_2O$	0.399g.	100 mls	10 mls
$CoCl_2 \cdot 6H_2O$	0.357g.	1000 mls	1 ml
$NaMoO_4 \cdot 2H_2O$	1.815g.	1000 mls	1 ml
NaEDTA·2H2O	0.750g.	100 mls	10 mls
$ZnCl_2$	0.818g.	1000 mls	1 ml

Weigh out the amounts, dissolve separately in listed amounts of RO water, pipet out the listed amounts into a 250 ml volumetric flask and bring volume up to the 250 ml mark with RO water.

Stock Spike #3 of 5

$MgSO_4 \cdot 7H_2O$ 3.675g.
Dissolve in 200 mls of RO water and bring up to 250ml volume in a volumetric flask.

Stock Spike #4 of 5

K_2HPO_4 0.261g.
Dissolve in 200 mls of RO water and bring up to 250ml volume in a volumetric flask.

Stock Spike #5 of 5

$NaHCO_3$ 3.750g.
Dissolve in 200 mls of RO water and bring up to 250ml volume in a volumetric flask.

Procedure for Liquid AAM Preparation

The 250 ml stock solution spikes used for AAM should be prepared quarterly. (The liquid AAM consists of 90 mls RO water and 1 ml of each of the 5 above mentioned spikes and the total volume brought up to 1 liter volume with more RO water.) Generally 16 liters are prepared at a time using a specified four liter graduated cylinder. Each of the five stock solutions is filter sterilized in to one of the RO water aliquots, using a 20cc syringe filled to the 16cc mark, and a 0.2um Acrodisc filter disc. The water and filter sterilized stock solutions are subsequently poured into a prepared cubitainer. The pH of the total amount prepared is adjusted to between 7 and 7.5 with a couple of drops of 1 N HCl. An aliquot is submitted for chemistry analysis (approx. 75 mls) in a labeled, 125 ml Nalgene bottle. A Sample Data Sheet is completed and submitted with sample (See attached AAM Sample Data Sheet). The AAM is stored in a cubitainer that has been rinsed with 10% HCl then RO rinsed and labeled as AAM and with a chemistry number. The chemistry number consists of the beginning ME (the specific code for media), the two digit week number of the year, and the 800 number for nutrients (see General S.O.P.). The cubitainer is stored in the lab 250 refrigerator (approx. 4°C).

Solid AAM Agar

Constituents
3.0g. of agar
250 mls of liquid AAM

The antibiotic spikes (below) are optional and are only used to reduce contaminants associated with the algal cells. If these are included they are not added until the above constituents have been autoclaved.

0.5 ml of Penicillin G spike
0.5 ml of Chloramphenicol spike
0.5 ml of Streptomycin sulfate spike
0.25 ml of micronutrient spike

*Note: Only revive ATCC cultures on 1% AAM agar without antibiotics.

Optional Antibiotic and Micronutrient Stock Spike Solutions

Penicillin G Spike
0.40g. penicillin G in 100mls RO water (=8ppm)

Chloramphenicol Spike
0.02g. chloramphenicol in 100mls RO water (=0.4ppm)

Streptomycin sulfate Spike
0.10g. streptomycin in 100mls RO water (=2ppm)

Micronutrient Spike
Stock Spike #2 of 5 liquid AAM spike (100mls removed then filter sterilized, see below)

Autoclave four reagent bottles at 121°C for 15 minutes and allow to cool before filtering the antibiotic or micronutrient solutions in to them. The above antibiotic and micronutrient spikes are separately filter sterilized using 100cc syringes and 0.2 um Acrodisc$_{T.M.}$ filter discs. These spikes should also be made up quarterly and they are stored in the refrigerator in lab 250 with the liquid AAM spikes.

Procedure for Solid AAM Agar

The AAM agar, for monthly isolates, is prepared by adding 3.0 grams of agar to 250 mls of liquid AAM. In a 500 ml acid bottle, with the cap loosely screwed, 250 mls of liquid AAM and 3.0g. agar (without antibiotic and micronutrient spikes) are autoclaved for 15 min. at 121°C. This is allowed to cool slightly (not solidify) before the plates are poured or the filter sterilized antibiotic and micronutrient spikes are added (see Constituents). When antibiotic spikes are added the media should be mixed well before the plates are poured. Approximately 25 mls per petri plate is poured into each of 10 sterile petri plates and allowed to cool and solidify. These poured plates are then stored in the empty, plastic bag that they came in. The bag of poured plates is kept in the refrigerator in room 274 until a monthly isolate plate needs to be streaked.

Materials

For a list of materials that are not standard laboratory equipment and are specific to this test see the Selenastrum capricornutum 96-Hour Static Chronic Toxicity Bioassay Protocol, located in the gray notebook in office 289.

TEST PROCEDURE

Setting-up the Test

Preparation for this test takes place the day before the test is to be Put-on. This consists of: labeling the glassware, filling out the paper work, and acclimating the AAM diluent and alleged toxicant to room temperature.

For a standard test, thirty-nine clean, sterilized and foam plugged, 125ml Erlenmeyer flasks are necessary (see Dishwashing S.O.P.). A standard test incorporates eleven concentrations at the following intervals:

80% toxicant- 60%- 30%- 10%- 6%- 3%- 1%- 0.6%- 0.3%- 0.1%- 0.01%

Each concentration is performed in three replicates. There are also three positive and three negative controls.

(11 concentrations)(3 replicates) = 33 + 6 controls = 39 flasks.

Each flask is labeled with the concentration and beginning at the 80% concentrations and proceeding through the lower concentrations the flasks are labeled one through thirty-three. The first flask (80% concentration, replicate number one) is marked with the chem. number, and the test code (see General S.O.P.). The positive controls are designated with the numbers 1,2, and 3. Each of these three flasks has "pos." and "Zn^{+}".

The negative controls are labeled "neg." 1,2, and 3. If more than one test is put-on in a day, only one set of controls are needed. The algal bioassay cover sheet and seven data sheets (7 data sheets used in a standard test each of which contains results of two test concentrations) can begin to be filled-out. The Test Code, Test Water, Chem. No., date sampled and date eluted can all be filled in (see example cover sheet). The data sheets receive: Test Code, Test Water, Chem. No., treatment, species, and concentration spikes (see example data sheet). For a more complete explanation of sample identification and designations, see General S.O.P..

Both the AAM and test sample need to be removed from their cold storage locations so that both equilibrate to room temperature before the test is put-on. This prevents algal temperature shock from occurring when the inoculum is added. The test sample (i.e. eluate, ground water, or surface water) and the AAM diluent are taken from the refrigerators (sample from vented refrigerator in lab 250 or from 159C, and AAM from refrigerator in lab 250). The test sample is placed in a hood in lab 250. The AAM is either left in the cubitainer and put on a counter in lab 250 overnight, or is dispensed into the flasks according to the dilution scheme on the back of the cover sheet (see example cover sheet). The dispensing of the AAM can be done the day before the test or the day the test is put-on.

Putting-On the Test

Putting-on the test involves: dispensing the test sample and AAM (AAM if not already dispensed); preparing, calculating, and dispensing the inoculum; recording initial pH measurements of high and low concentrations; checking test incubation conditions; and completing the paper work.

The AAM is dispensed first using a 250ml buret labeled "AAM Only" (or any clean buret), located in lab 250. The lower concentrations require the additional use of 1ml and 5ml automatic pipets. These are used to subtract small volumes that can not be measured as accurately with the 250ml buret. It is not necessary to dispense the AAM under a hood. The test sample is dispensed under a hood in lab 250 from a clean 250ml buret and appropriate automatic pipets for the lower concentrations (see example below).

% concentration	mls sample	mls diluent (AAM)
80	40	10
60	30	20
30	15	35
10	5	45
6	3	50-3.0=47
3	1.5	50-1.5=48.5
1	0.5	50

An extra 1% concentration is prepared in a medium size beaker and used for the 0.1 and 0.01% concentrations.

0.6	0.3	50
0.3	0.15	50
0.1	5mls of 1% conc.	45
0.01	0.5ml of 1% conc.	50

The negative and positive control flasks each receive 50 mls of AAM. The positive control flasks also receive 1 ml each $ZnCl_2$ spike solution. This solution is prepared as described below.

$ZnCl_2$ Stock Spike Solution

Weigh out 3.86 g. $ZnCl_2$ and place in a 500 ml volumetric flask before bringing the volume up to 500 mls with RO water. Pipet one ml of this solution into 1 L. volumetric flask and bring up to 1 L. volume with RO water. This is the solution used for spiking the positive control flasks. (One ml in 50 mls AAM produces approximately a 74ug/L. Zn^+ solution.)

Measuring the Initial pH

Initial pH measurements are taken of the high (80%) concentration and low (0.01%) concentration. The pH meter is calibrated before readings are taken (see QA/ QC S.O.P.). The pH measurements are read from a pH meter located under a hood in lab 250. These pH values are recorded on the Cover Sheet.

INOCULUM

Inoculum Preparation

The *Selenastrum capricornutum* inoculum is prepared from a three to seven day old stock culture obtained from the algal culture room, room 274. In lab 250, the inoculum is prepared the day the test is put-on, after the sample and diluent have been dispensed into the 125ml Erlenmeyer flasks. The stock culture is poured into a clean, sterilized, foam plugged 500ml Erlenmeyer flask. This is diluted with RO water to around the 200 to 250 mark. Before this can be counted, a 1% saline background count must be determined on the Coulter Counter.

Coulter Counter Operation

The Coulter electronic particle counter needs to be turned on fifteen minutes before counts can be made. Turn on the sampling stand, the mean cell volume recorder, and the particle counter. Change the mean cell volume recorder to corrected count and check all settings with those listed on the index card taped to the particle counter.

Settings for ZM# 30987 Coulter Particle Counter, specified for algae toxicity bioassay.

Full Scale
Current =700 x 1 Auto
Lower Threshold = 5 Upper Threshold 99.9
KD = 15.7
Dia.= 4.76u Vol.= 56.5u^3
Manometer = 500

This equipment requires training from qualified HWAT or Coulter personnel and any questions as to its operation should be checked with the machine's operation manual located in the filing cabinet in office 287 or with the proper staff members. Any operation processes mentioned here are not intended to be inclusive of all procedures that must be followed to correctly operate this equipment. Monthly calibration is discussed in the QA/QC S.O.P.

1% Saline Preparation

First, a 20% NaCl solution is made by mixing 400g. NaCl with 1850mls of RO water. Using a 200ml graduate cylinder, 200mls of the 20% solution are mixed with 3800mls of RO water to obtain the 1% solution which is then filtered. The 1% saline is prepared and vacuum filter sterilized four times through a 0.22um filter. The filtered 1% saline is poured into designated saline bottles with Oxford pipettor screw cap lids capable of dispensing 10mls (generally set at the 9ml mark for Coulter Counter dilutions).

Background Counts

The Coulter Counter is "flushed" before determining a background count. This is accomplished by first placing a tube with saline in the Coulter sampling stand and raising the platform until the electrode is well submersed into the saline solution. Close the glass door and turn the lower knob to the fill position. Then, turn the upper knob to the reset position and hold for approximately 10 to 30 seconds before turning this knob to the count position. Then immediately turn the lower knob back to the closed position. Check the aperture for clogs and if necessary remove debris with small brush. Check the "waste" and "fill" jars in the back of the Coulter Counter (see Coulter Counter Operations Manual). Using another Coulter tube with 9mls of saline, a background count is determined. Place tube on stage and position the electrode in the saline solution. Close the door. Turn the top knob to the reset position until the counts read zero. Then turn this knob to the count position and wait until the count is completed. This is repeated five times. The five counts are averaged and this is recorded as the background count.

Counting the Stock Culture For Inoculum Use

Now, the stock culture to be used for the inoculum can be counted. Nine mls of the 1% saline solution is dispensed into Coulter tubes. One milliliter of RO water diluted stock culture is swirled and then pipetted into a Coulter tube containing 9mls of 1% saline (dispensed from saline bottle with Oxford 10ml capacity pipet lid set at 9mls). This is mixed, by aspirating with the pipet, before 1ml is pipetted out into another tube containing 9mls of saline, so that a 1:100 dilution of stock culture has been achieved. The Coulter Counter further dilutes this by 1:2, so that upon counting a total dilution factor of 1:200 is reached. If the counts are greater than 6,000, then more RO water is added to the stock solution, and another 1:200 dilution made and another count taken. When the stock culture is dilute enough so that the counts are between 4,000 and 6,000 (this ensures that the inoculum volume will be close to 0.5ml, our standard inoculum volume) and the MCVs are greater than 30, (our lower MCV limit) the stock culture is acceptable for inoculum use. Seven counts are taken and recorded with their corresponding MCVs on the reverse side of the Cover Sheet. The highest and lowest counts are not used to determine the amount of inoculum to be added to each flask to create a 10,000 cells/ ml suspension (i.e. in 50ml test volume the inoculum must distribute 500,000 cells per flask). The five intermediate counts are entered into a program on the HP calculator to determine the amount of stock culture to be inoculated into each flask, the average MCV (Mean Cell Volume) and the milligrams/ liter dry weight of algae (see Inoculum Calculator Instructions).

Inoculum Calculator Instructions

(See Appendix for Programming Calculator)

1. Turn on Calculator

2. Press "f" (second function) and then "clear Σ".

3. Enter background number, then press "STO" and then 6.

4. Enter test volume amount in mls (50) and press "STO", then 7.

5. MCVs and cell counts

		Display
(1 of 5)	MCV no. ENTER	
	enter count and then $\Sigma+$	1.000
(2 of 5)	MCV no. ENTER	
	enter count and then $\Sigma+$	2.000
(3 of 5)	MCV no. ENTER	
	enter count and then $\Sigma+$	3.000
(4 of 5)	MCV no. ENTER	
	enter count and then $\Sigma+$	4.000
(5 of 5)	MCV no. ENTER	
	enter count and then $\Sigma+$	5.000

6. Press "f" then A
The value displayed represents the volume (ml) of stock culture corresponding to 500,000 cells (10,000 cells/ml) to be added to each flask as an inoculum. Record this value and those generated in steps 7 and 8 (below) in the inoculum portion of the Algae Cover Sheet.

7. Press R/S
The value displayed is the average mean cell volume

8. Press R/S again
This value is the milligrams / liter dry weight algae in the inoculum.

*Note:
MCV x Dilution Factor x Counts x 3.6x10-7 = mg./L. dry weight

Dispensing the Inoculum

The diluted stock culture that is to be used for inoculating the test flasks is placed on a magnetic stir plate and a small stir bar is placed in the culture flask. The stir plate is set on low, number 2 setting. Using a 1ml adjustable pipet set to the volume designated in step 6 (above), each test flask containing 50mls is inoculated with 500,000 algae cells (i.e. 10,000 cells/ml).

All the flasks are put onto a gray, Rubbermaid$_{T.M.}$ cart and taken to room 274 for incubation.

Checking Environmental Parameters in Rm 274

The lights are checked with a light meter located in room 274. All general locations on the shaker table where flasks are to be placed should be checked to make sure all areas are in the acceptable light incubation range 360-440 foot candles. If the light meter reads greater than 440, aluminum foil is placed on areas of the bulbs until the range is acceptable. If the light intensity is too low, then the aluminum foil is adjusted to allow more light to reach the shaker table or the fluorescent bulbs are changed and the lights are checked again. The shaker table is turned on and the revolutions in a 15 second time span are counted. This number is multiplied by 4 to get the rpms (revolutions per minute). If this is not between 90 and 110, the rate is turned up or down accordingly. The rpms are then checked again. Both the light range and rpms of the shaker table are recorded in room 274 in the test incubation conditions book. The flasks are arranged on the shaker table in numerical order from front to back, left to right, and left to incubate for 96 hours.

The Test Preparation portion of the cover sheet is filled in with the initials of the technician that executed each specific procedure. The cover sheet and attached data sheets are hung on the wall in lab 250 on a hook that corresponds to the day the test is to be counted. Example: if the test is put-on Thursday, then the paper work would be placed on the Monday hook (to be counted 96 hours later).

Counting the Test

Sampling

The test is counted after 96 hours of incubation in room 274. The sampling procedure for counting the test is initiated by taking one rack of Coulter tubes per test to be counted and a one milliliter automatic pipet with disposable tips to room 274. The flasks are examined for precipitates that may have formed during the test incubation. If precipitates are present, the test must be counted by chlorophyll *a* fluorescence (see procedure below) and electronic particle counting. If a test contains precipitates and is also a highly colored sample, so that fluorometry would be ineffective, it must be counted directly using a hemocytometer and a microscope. Tests containing precipitates are noted on their cover sheets.

A rack of Coulter tubes is used for each individual test that is to be counted in a day. There are forty tubes in a rack and usually thirty-three flasks per test. That leaves seven extra per rack, six of which are reserved for the positive and negative controls.

The left, front tube is labeled with the chemistry number of the test and is by convention designated as the number one position, and is used for the number one flask (80% concentration, replicated number one). Each flask is swirled before sampling. One milliliter is pipetted from this flask and dispensed into this Coulter tube. Proceeding to flask number two, a one milliliter aliquot is removed from the flask and pipetted into the second tube in the first row. This process is repeated for each flask. Aliquots from flask one through ten are located in the first row, eleven through twenty in the second row and so on. Using this convention, each concentration occupies a designated spot in the rack. For example, on a standard test the 1% concentration replicates, flask numbers 19, 20 and 21, are located in the last two positions of row two and the first position of row three in the tube rack. Pipet tips are changed after each test, as long as sampling is from low algae (less green) to high algae and the test is to be terminated that day, and also between the negative and positive controls. Obviously aberrant flasks within a test should receive new tips. Flask numbers one and thirty-three (highest and lowest concentrations) from each test, along with the Coulter tubes, containing test sample, are taken via gray cart to lab 250 for counting and final pH measurements.

Counting

The Coulter particle counter should be turned on fifteen minutes before its intended use. The electrode should be "flushed", the settings checked, counts set on corrected, and a 1% saline background count determined as explained in the preceding inoculum section. The background count is recorded on each data sheet. Nine milliliters of saline are dispensed into each tube containing one milliliter of sample (i.e. 1:20 dilution upon counting).
The dilution factor (20, 200 or 2000) is recorded on the data sheet as the concentrations are being counted. Three counts are taken of each replicate tube. The counts and corresponding MCVs are recorded on the data sheet under the appropriate concentrations (see example data sheet). If the counts are too high (above 35,000), a dilution must be made. This is accomplished by pipetting one milliliter of the 1:20 dilution into another tube and dispensing 9 mls of 1% saline into it, thereby creating a 1:200 dilution. Further 10x dilutions (to 1:2000) may be necessary at times. Once the counts are sufficiently high enough at a particular concentration to require additional diluting of 1:200, the rest of the succeeding tubes (i.e. lower concentrations) will probably require a dilution factor of 200 also. This is carried out by transferring one milliliter of each 1:20 diluted tubes remaining to be counted into corresponding positions on a parallel rack containing Coulter tubes. The 9mls of 1% saline are not added until just prior to being counted, so that settling does not occur causing erratic results.

The rest of the tubes are then counted and recorded on the data sheet with the new 200 dilution factor noted in the appropriate site. The positive and negative controls are counted and recorded on a separately labeled data sheet. The positive control usually only requires a 1:20 dilution where as the negative control generally needs to be diluted 1:200. Flask number 1 (high concentration) and flask number thirty-three (low concentration) from each test are used to obtain final pH measurements. The pH meter located in a hood in lab 250 is calibrated before these are measured and recorded on the Cover Sheet. The technician responsible for sampling and counting, places their initials in the appropriate lines of the Cover Sheet (see example).

Subculture

If the counts at any given concentration are <25,000 cells/ml or <0.45 mg/L dry weight (approximately <1500 counts at a dilution factor of 20), then a subculture to determine lethality (algicidal vs. algistatic results) must be performed. This is accomplished by removing 0.5ml from each of the three replicates and placing them into a 500ml Erlenmeyer flask containing 100ml of fresh AAM. This is incubated for seven days in room 274. The flask and a data sheet are labeled with the sample identification and "subculture" concentration specification. The subculture data sheet is hung in lab 250 on the hook labeled with the day it is to be counted. If the yield after seven days is <25,000 cells/ml or <0.45 mg/L dry weight, the solution is algicidal. This is calculated at present on the APPLE Computer (training by HWAT personnel required).

Calculations

The data obtained from the algae bioassay toxicity test using the Electronic Coulter Particle Counter is entered in to a Lotus 123 Program developed in house. This program is extensive and requires training from HWAT personnel. The program calculates the mg/liter dry weight in each flask using the equation:

dilution factor x cell counts X MCV X 3.6×10^{-7} = *S. capricornutum* (mg/L dry weight)

Each flask is treated as a single data point. The test yield for each flask is normalized and compared directly to the negative control yield, example: 80% toxicant contains 20% AAM, so the expected "no effect" response would be 20% of the negative control value. This is used to determine the percent effect. Each data point is then transformed to give log percent effect and log toxicant concentration, and these are input into a Stat Graphics Program (training required) and an EC_{50} value graphically generated.

Chlorophyll *a* Fluorescence

Any test in which a precipitate has formed in the test solution must have a chlorophyll *a* fluorescence determination performed. The fluorometer is located in lab 250. Six milliliters per replicate are required to execute this procedure. A five ml automatic pipet set at 3ml is used to remove two 3ml aliquots from each flask, which are dispensed into a separate Coulter tube rack in the same order and corresponding locations as described for Coulter Counter sampling. Some technicians will combine the sampling by removing two 3.5ml aliquots from each flask (totaling seven mls, one of which is used for Coulter counting and six for fluorometry determination). The fluorometer needs to be turned on fifteen minutes before its intended use. This equipment requires training from qualified HWAT personnel. Measurements of chlorophyll *a* fluorescence are made after first adjusting the fluorometer with a RO water blank. This is accomplished by placing 6 mls of RO water into an appropriate fluorometer test tube and putting this tube into the fluorometer chamber. The "blank" knob is then adjusted until the scale reads zero. The RO water is discarded and 6 mls of test solution are placed into the fluorometer test tube and this tube placed in the chamber. With the range set on "auto", approximately thirty seconds are required before the scale can be read. The machine will automatically cycle through the series settings. If the reading is off scale and the filter setting is X100, change the filter setting to X1 by flipping the pointer switch. If after another thirty seconds the reading is still off scale, the sample must be diluted 1:2 with RO water before the results can be read. Once the reading remains on scale; the filter setting, series, upper scale reading, and dilution factor (if any) are recorded on a test labeled fluorometry data sheet (see fluorometry data sheet example). The series being used is indicated by a red light next to the series numbers on the light board located on the front panel of the instrument. The filter setting is recorded from the pointer switch setting and the upper scale reading is from the scale needle using the top scale. Filter settings of 1X in conjunction with series settings of min. sens. or X3.16 are not to be used. One reading is taken and recorded for each replicate (three per concentration). The RFU's (relative fluorescence units) are calculated using the HWAT's group HP calculator (see instructions below). This program is completed on each replicate reading.

RFU Calculator Instructions

1. Turn on calculator, press "g" then blue "clx" button.

2. Enter series # then press "STO", then press 1.

3. Enter filter setting # then press "STO" then press 2.

4. Enter upper scale reading #, then press "ENTER".

5. If dilution factor does not equal 1, key in dilution factor and pres "x" (multiplication key).

6. Press "f" (second function) then "B".

7. Calculator stops and displays RFU, this is recorded on the fluorometer data sheet.

8. If you want to change the series or the filter setting, key in the new number and press STO 1 or STO 2 respectively and proceed with step 4 for next calculation.

The RFU's are recorded on the fluorometry data sheet. The negative control RFU's are averaged, then the percent effect is calculated using the Algal D/E Calc program on the APPLE computer (training is required). The transformed percent effect (TPE), $\log_{10}$ toxicant, and $\log_{10}$ effect are calculated on a LOTUS 123 spread sheet from the disk labeled: Algae, SOS test programs (training also required).

Hemocytometer Counting

Samples that contain precipitates and are too dark or colored for fluorometry, must be counted with the use of a hemocytometer counting chamber and a microscope. This allows for direct examination of the cells. Appropriately place the cover slip over the grids on the hemocytometer slide. Use a capillary pipet to remove algal cells from a well mixed flask. Pipet an aliquot into the hemocytometer slide channel chamber. Be sure that no air bubbles remain in the chamber. Count the cells in the nine diagonal grids under the microscope using high power (2X). Calculate the cells/ml using the following equation:

cells/ml=
(# of cells in 9 grids) / (9 grids) X (25 grids total) X 10,000

Once the cells/ml are calculated the counts versus concentration can be plotted to determine the EC$_{50}$ value.

APPENDIX--CALCULATOR PROGRAMMING INSTRUCTIONS

The calculations for the inoculum may be programmed onto a hand held calculator for convenience. Our lab uses a Hewlett-Packard 11C programmable calculator, to which the following instructions apply.

Turn on calculator. Press g P/R to get into programming mode. The PRGM annunciator will appear in the display. Press the keys GTO, ., and 000 in sequence. This sets the calculator at line 000 without affecting other programs already in the calculator. If you want to get rid of all previous programs in the calculator, press f CLEAR PRGM.

Key in the keystrokes listed in the KEYSTROKES column. The calculator will display what is in the DISPLAY column. The function of each set of keystrokes is explained in the DOCUMENTATION column.

More instructions for programming may be found in the HP-11C Owner's Handbook and Problem Solving Guide.

KEYSTROKES	LINE NO	DISPLAY	DOCUMENTAT'N
f LBL A	001	42,21,11	Names and defines start of program
g $\bar{x}$	002	43,0	Finds mean of cell cts
RCL 6	003	45,6	Recalls bkgrd
-	004	30	Subtracts bkgrd from cell ct
1/x	005	15	Findsreciprocal of cell ct
1	006	1	Multiplies
0	007	0	10,000
0	008	0	by reciprocal
0	009	0	of corrected
0	010	0	counts =
x	011	20	(cts-bkgrd)
2	012	2	divides

0	013	0	by
0	014	0	200
÷	015	10	
RCL 7	016	45,7	Recalls test vol
x	017	20	x test vol
R/S	018	31	Stops, displays mls inoc
x><y	019	34	mean of MCV's
R/S	020	31	Stops, displays MCV
1	021	1	Multiply
EEX	022	26	by
4	023	4	10,000
x	024	20	
3	025	3	Multiply
.	026	48	by
6	027	6	3.6
EEX	028	26	x
CHS	029	16	10
7	030	7	-7
x	031	20	Displays mg dw
g RTN	032	43,32	Ends program

TEST CODE: PH 060989 A

TEST WATER: Hot spot-1

CHEM NO. PHN 30009

DATE SAMPLED: 011589

DATE ELUTED: 030789

	high conc.	low conc.
INITIAL pH:	7.9	7.1
FINAL pH:	9.9	9.2

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 04

INOCULUM:

Selenastrum c. ALGA

~10^4 CELLS/ML

0.1698 MG/L DRY WT.

47.2 MCV

0.573 MLS INOCULUM

TEST PREPARATION:

TEST WATERS: WB AAM BATCH MEN23800

DILUTION MEDIA: WB

INOCULUM: JUN

INOCULATED: JUN

SAMPLED: MB

COUNTED: MB

TEST/CONTAINER VOLUME: 50/125

REPLICATE FLASKS: 11 concentrations @ 3reps ea.

DILUTION RANGE: 80% to 0.01%

NOTES: Poison Hill site sample eluate

TEST WATER: Ht Spot-1 TEST CODE: PH060989A

TREATMENT: centrifuged & .45 um filtered SPECIES: [illegible]

CHEM. #: PHN30009 SPIKE: 80%

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
04	20	84	1	1348	37	1401	36	1411	36
			2	1798	41	1632	43	1700	43
			3	1541	39	1590	39	1601	39
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 60%

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
04	200	84	1	7801	40	8011	39	8100	40
			2	10019	36	9816	35	10988	36
			3	8319	42	8412	41	8390	41
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SAMPLE DATA

Lab 250
Location

technician's initials
Recorder

MO DA YR ☐☐☐☐☐☐ ↙ date AAM prepared

Notes: AAM = algal assay medium

Date Received	
Date Output	

Initial pH	Final pH	Origin Code	Adjustment	Lab. No. Wk	Lab. No. No.	Sample Description	Matrix (L or S)	Metals	Organics	Nutrients	REMARKS	ICP Record #
	73	ME	N	23	800	aliquot II	L	X	O	O		

White & Pink: File Yellow & Green: Chemistry

Example Data Sheet
FLUOROMETRY

Test Code:________________ Test Water:________________________________

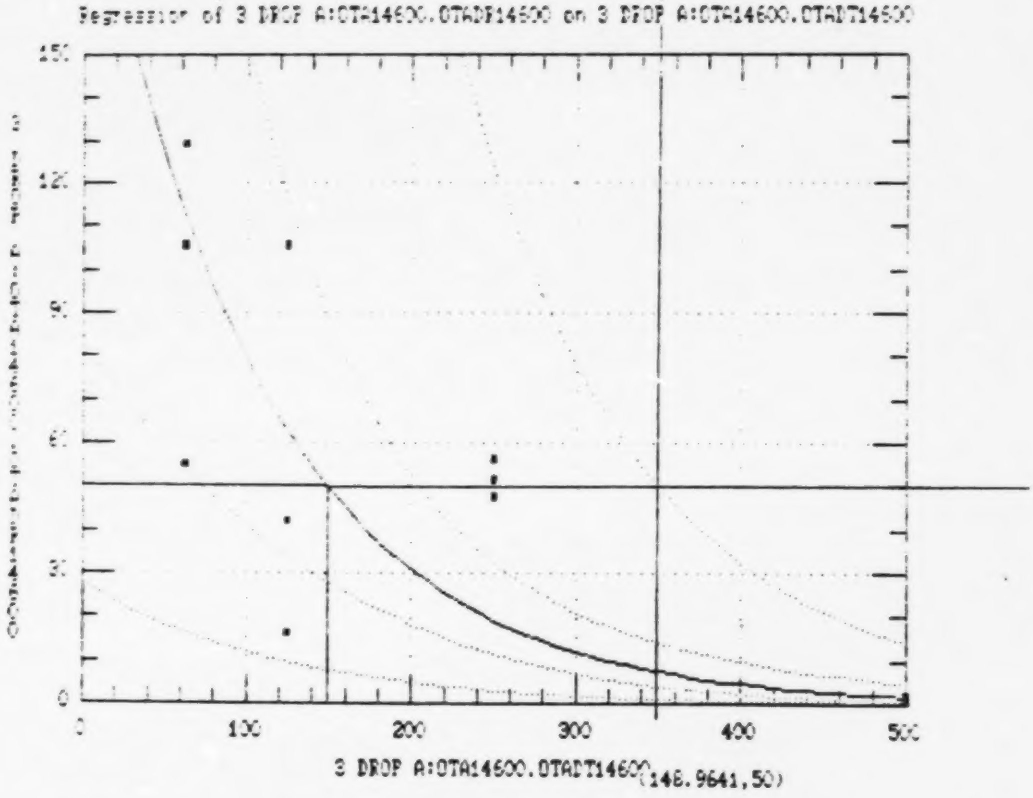
Chem. No.:________________ Technician:______________ Date:____________ Species:Selenastru

% Toxicant	Replicate	Series Setting	Filter Setting	Upper Scale Reading	Dilution of Sample	Calc. RFU'
80	1					
	2					
	3					
60	1					
	2					
	3					
30	1					
	2					
	3					
10	1					
	2					
	3					
6	1					
	2					
	3					
3	1					
	2					
	3					
1	1					
	2					
	3					
.6	1					
	2					
	3					
.3	1					
	2					
	3					
.1	1					
	2					
	3					
.01	1					
	2					
	3					
neg	1					
	2					

APPENDIX B

ANOVA TABLES AND REGRESSION GRAPHS

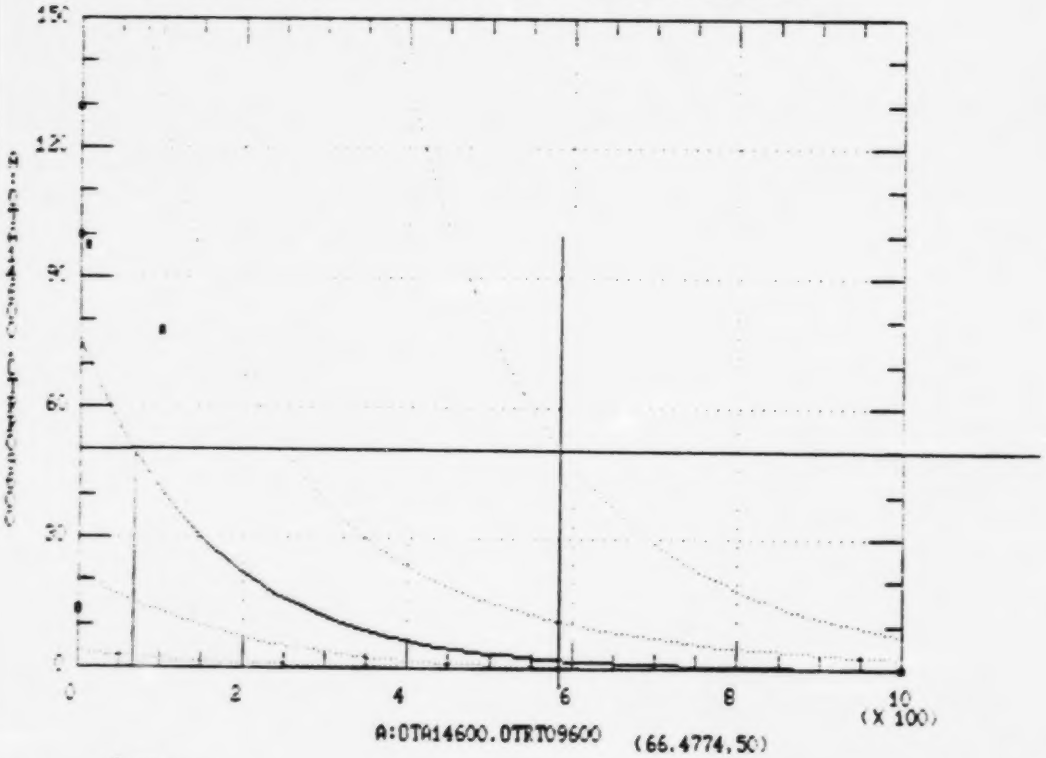
```
Regression Analysis - Exponential model: Y = exp(a+bX)
=========================================================================================
Dependent variable: 3 DROP A:OTA14600.OTIndependent variable: 3 DROP A:OTA14600.
-----------------------------------------------------------------------------------------
                                Standard              T                Prob.
Parameter       Estimate         Error              Value              Level
-----------------------------------------------------------------------------------------
Intercept        5.31139        0.397094           13.3756         1.0483E-7
Slope       -9.39401E-3      1.37827E-3           -6.8159         4.64943E-5
-----------------------------------------------------------------------------------------

                               Analysis of Variance
-----------------------------------------------------------------------------------------
Source                 Sum of Squares    Df   Mean Square    F-Ratio   Prob. Level
Model                       29.731805     1     29.731805  46.455137        .00005
Error                       6.4001114    10      .6400111
-----------------------------------------------------------------------------------------
Total (Corr.)               36.131917    11

Correlation Coefficient = -0.907121          R-squared =  82.29 percent
Stnd. Error of Est. = 0.800007
```



GLYORAL - DEFINITIVE TEST
EC_{50} = 148.96 (149.0) mg/L
(0-350)

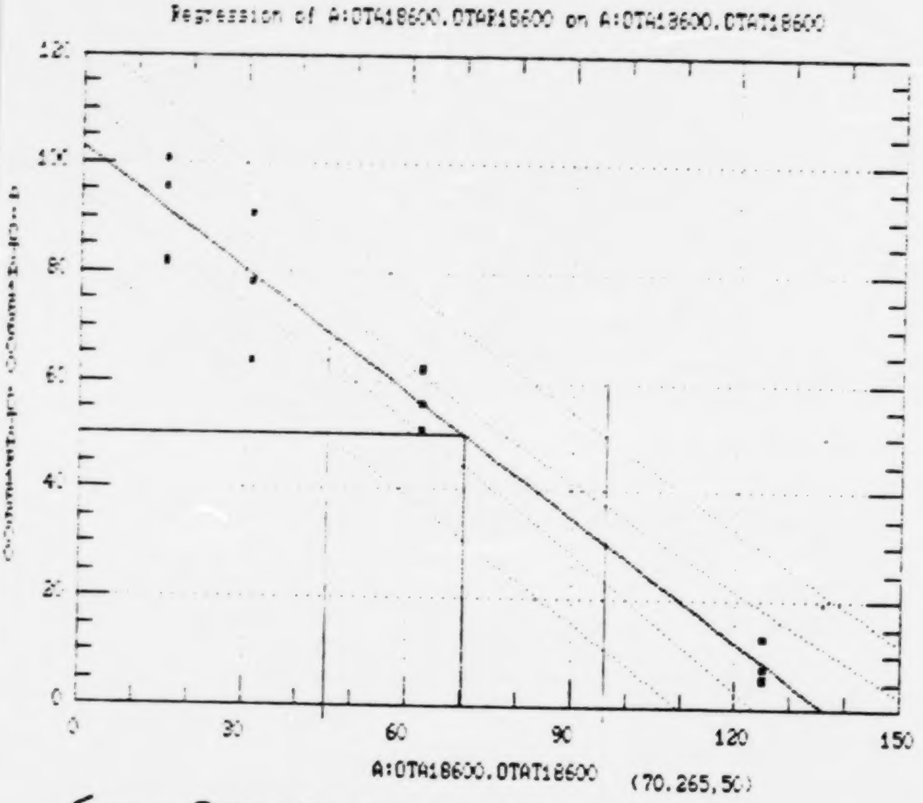
```
gression Analysis - [redacted] model: Y = exp(a+bX)
=============================================================================
pendent variable: A:OTA14600[redacted]ependent variable: A:OTA14600.OTRTO96
-----------------------------------------------------------------------------
                              Standard              T              Prob.
rameter        Estimate         Error             Value            Level
-----------------------------------------------------------------------------
tercept         4.32085        0.451032          9.57991        6.63435E-4
ope          -6.1498E-3      1.09926E-3         -5.59449        5.00998E-3
-----------------------------------------------------------------------------

                         Analysis of Variance
-----------------------------------------------------------------------------
urce                Sum of Squares    Df  Mean Square    F-Ratio  Prob. Level
del                      30.420143     1    30.420143  31.298278       .00501
ror                      3.8877722     4     .9719430
-----------------------------------------------------------------------------
tal (Corr.)              34.307916    [redacted]

rrelation Coefficient = -0.941637          [redacted]
nd. Error of Est. = 0.985872
```

Regression of A:OTA14600.OTRRO9600 on A:OTA14600.OTRTO9600



GLYOXAL
RANGE-FINDING TEST
EC_{50} = 66.5 mg/L
(0-580)

```
gression Analysis - [redacted]del: Y = a+bX

pendent variable: A:OTA18600.[redacted]ependent variable: A:OTA18600.OTAT186

                              Standard              T              Prob.
rameter        Estimate         Error             Value            Level

ercept           103.14        4.06907           25.3474     2.09423E-10
ope           -0.756285       0.056493          -13.3872      1.03771E-7

                          Analysis of Variance

urce              Sum of Squares     Df  Mean Square     F-Ratio  Prob. Level
del                    12043.250      1    12043.250     179.218       .00000
ror                    671.98789     10     67.19879

tal (Corr.)            12715.238  [redacted]

rrelation Coefficient = -0.973217          [redacted]
nd. Error of Est. = 8.19749
```

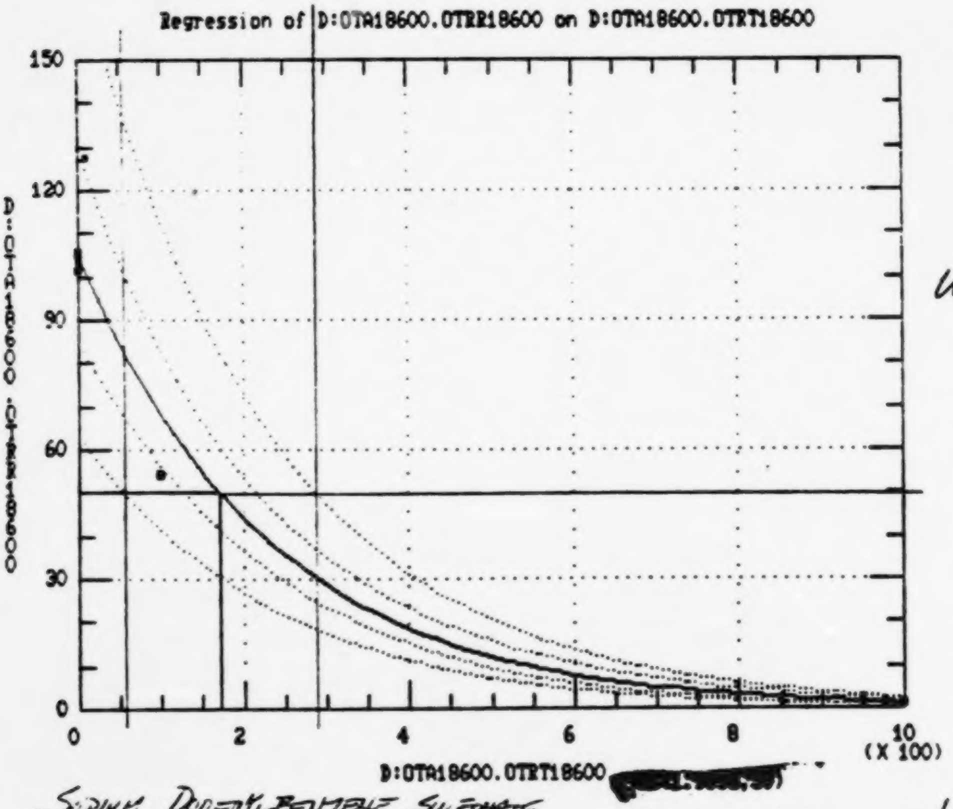


SODIUM DODECYL BENZENE SULFONATE
DEFINITIVE TEST
$EC_{50} = 70.3$ mg/L
(45-95)

RANGE-FINDING TEST: SDBS

```
Regression Analysis [redacted] Y = exp(a+bX)
------------------------------------------------------------------------------
Dependent variable[redacted]ependent variable: D:OTA18600.OTRT186
------------------------------------------------------------------------------
                                  Standard              T               Prob.
Parameter          Estimate        Error              Value             Level
------------------------------------------------------------------------------
Intercept            4.6517      0.0760193           61.1911        4.27196E-7
Slope           -4.30044E-3     1.85275E-4          -23.2111        2.04179E-5
------------------------------------------------------------------------------

                          Analysis of Variance
------------------------------------------------------------------------------
Source              Sum of Squares      Df   Mean Square    F-Ratio  Prob. Level
Model                     14.87531       1      14.87531   538.75652      .00002
Error                     .1104418       4      .0276104
------------------------------------------------------------------------------
Total (Corr.)            14.985748  [redacted]

Correlation Coefficient = -0.996308          [redacted]
Stnd. Error of Est. = 0.166164
```

05-01-89

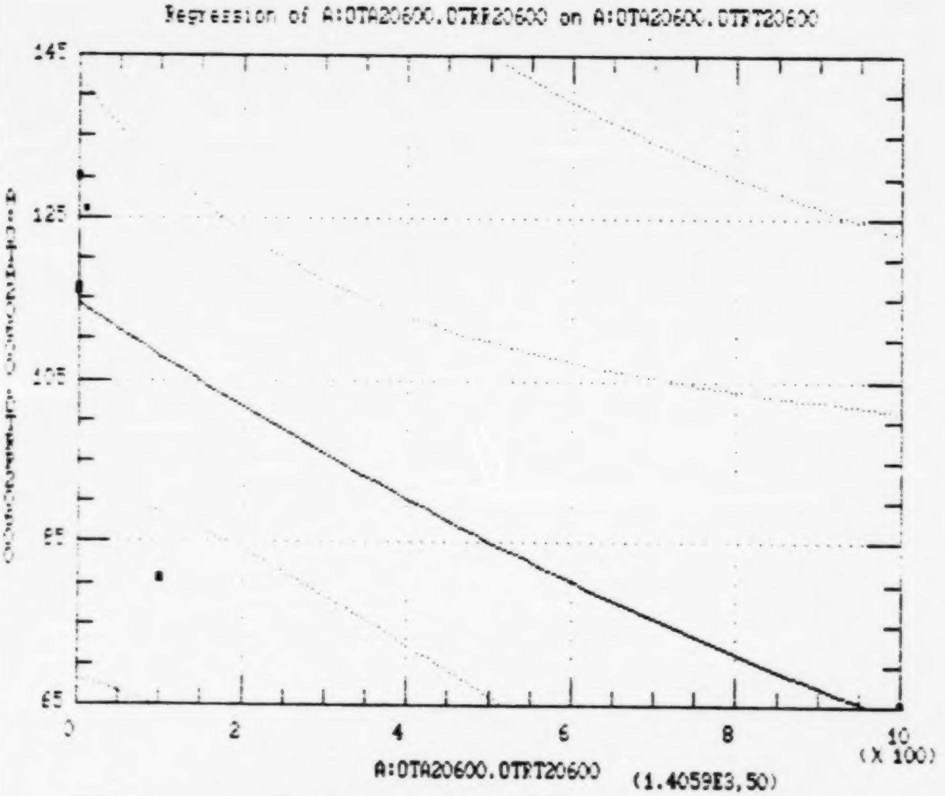


UNTRANSFORMED DATA

SODIUM DODECYLBENZENE SULFONATE
RANGE-FINDING TEST

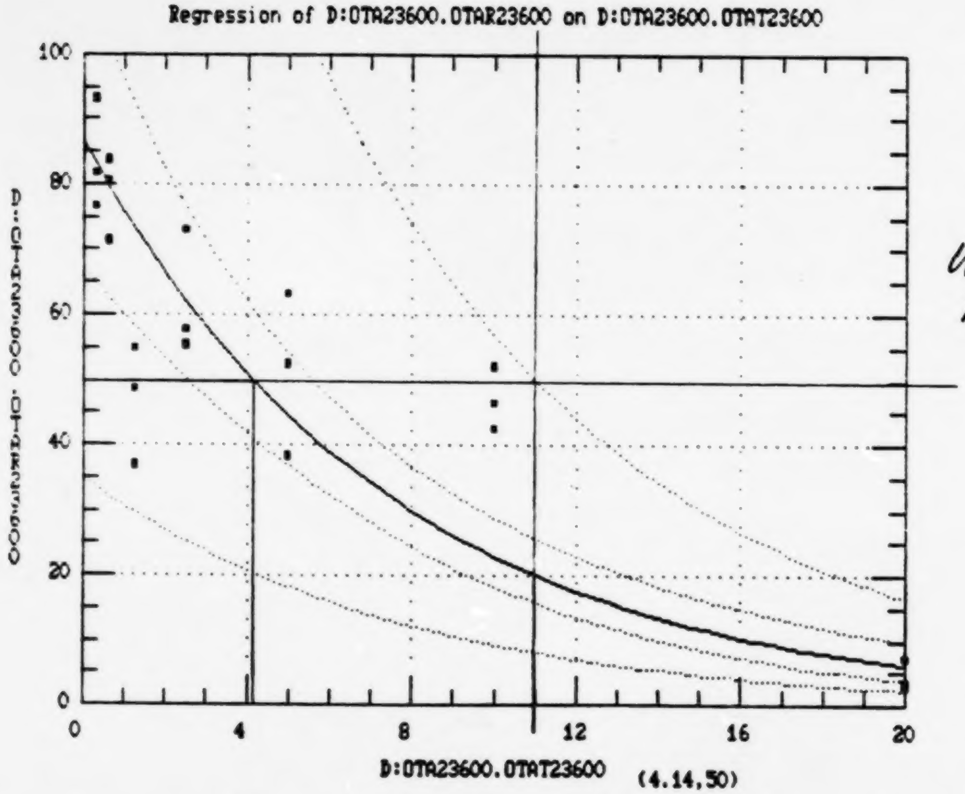
EC_{50} = 172 mg/L
(50-290)

```
ression Analysis - [redacted] odel: Y = exp(a+bX)

ependent variable: A:OTA20600.[redacted] dependent variable: A:OTA20600.OTRT206

rameter          Estimate       Standard           T             Prob.
                                  Error           Value          Level

ercept            4.73971       0.0771062        61.4695       4.19504E-7
pe           -5.88736E-4       1.87924E-4       -3.13284        0.0350889

                          Analysis of Variance

urce                   Sum of Squares    Df   Mean Square    F-Ratio   Prob. Level
del                          .2787928     1      .2787928   9.8147039        .03509
ror                          .1136225     4      .0284056

tal (Corr.)                  .3924153    [redacted]

rrelation Coefficient = -0.842884          [redacted]
d. Error of Est. = 0.16854
```



TETRA ETHYL AMMONIUM CHLORIDE
RANGE-FINDING TEST
EC$_{50}$ = 1406 mg/L
(NC1)

```
Regression Analysis - [redacted]Y = exp(a+bX)
===========================================================================
Dependent variable: [redacted]Independent variable: D:OTA23600.OTAT236
---------------------------------------------------------------------------
                           Standard            T              Prob.
Parameter     Estimate        Error          Value            Level
---------------------------------------------------------------------------
Intercept      4.46115      0.120311          37.08               0
Slope        -0.132638     0.0137838       -9.62279      9.74196E-9
---------------------------------------------------------------------------

                          Analysis of Variance
---------------------------------------------------------------------------
Source               Sum of Squares     Df  Mean Square    F-Ratio  Prob. Level
Model                     16.269709      1    16.269709  92.598050       .00000
Error                      3.338348     19      .175703
---------------------------------------------------------------------------
Total (Corr.)             19.608057  [redacted]

Correlation Coefficient = -0.910904          [redacted]
Stnd. Error of Est. = 0.419169
```

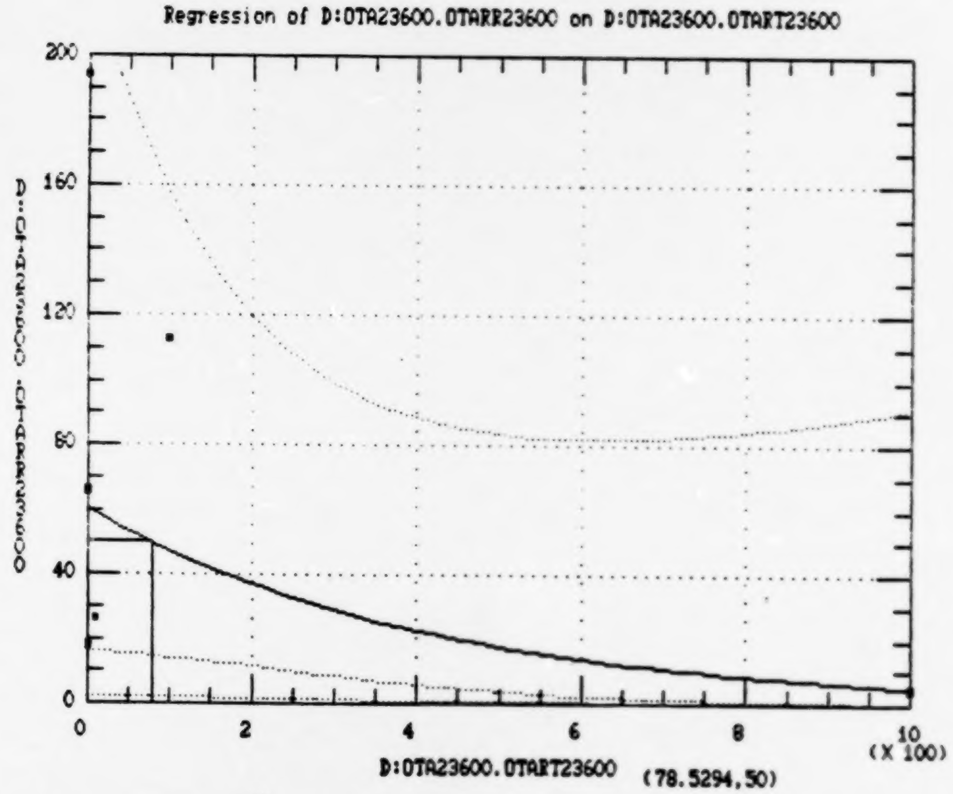


UNTRANSFORMED DATA

PHTHALIC ANHYDRIDE
DEFINITIVE TEST

EC_{50} = 4.1 mg/L
(0-11)

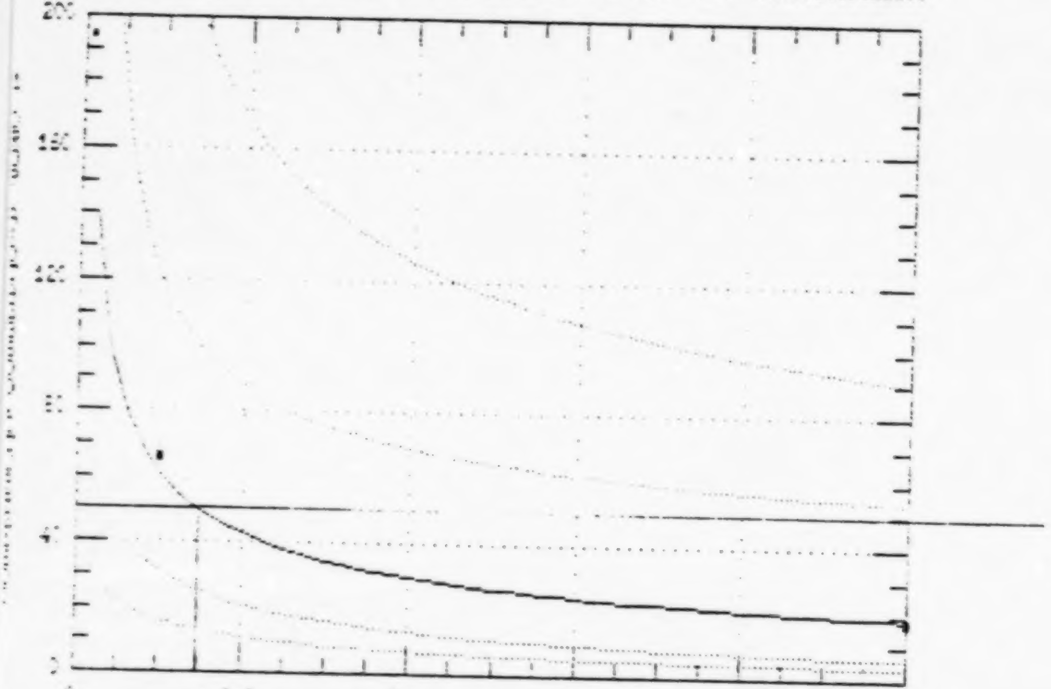
```
Regression Analysis -[redacted]model: Y = exp(a+bX)

Dependent variable:[redacted]ependent variable: D:OTA23600.OTART23

                              Standard           T            Prob.
Parameter      Estimate        Error           Value          Level

Intercept       4.10427       0.472631        8.68388     9.67932E-4
Slope       -2.44809E-3      1.15191E-3     -2.12526        0.100744

                          Analysis of Variance

Source              Sum of Squares    Df  Mean Square    F-Ratio  Prob. Level
Model                    4.8205259     1    4.8205259  4.5167297       .10074
Error                    4.2690409     4    1.0672602

Total (Corr.)            9.0895668   [redacted]

Correlation Coefficient = -0.728242        [redacted]
Stnd. Error of Est. = 1.03308
```



PHTHALIC ANHYDRIDE
RANGE-FINDING TEST
EC_{50} = 78.5 mg/L
(N.C.I.)

```
gression Analysis - Multiplicative model: Y = aX^b
================================================================================
pendent variable: 1 DROP A:OTA23600.OTIndependent variable: 1 DROP A:OTA23600.
--------------------------------------------------------------------------------
                                Standard              T             Prob.
rameter        Estimate          Error              Value           Level
--------------------------------------------------------------------------------
tercept*        2.91799        0.0842339          34.6415        0.0183723
ope            -0.513436       0.0283365         -18.2957        0.0347616
TE: The Intercept is equal to Log a.
--------------------------------------------------------------------------------

                          Analysis of Variance
--------------------------------------------------------------------------------
rce                  Sum of Squares     Df  Mean Square    F-Ratio  Prob. Level
del                         2.85004      1      2.85004  334.73124        .03475
ror                        .0085144      1     .0085144
--------------------------------------------------------------------------------
tal (Corr.)               2.8585572      2

rrelation Coefficient = -0.99851           R-squared =  99.70 percent
d. Error of Est. = 0.0922736
```

Regression of 1 DROP A:OTA23600.OTURT21600 on 1 DROP A:OTA23600.OTURT21600

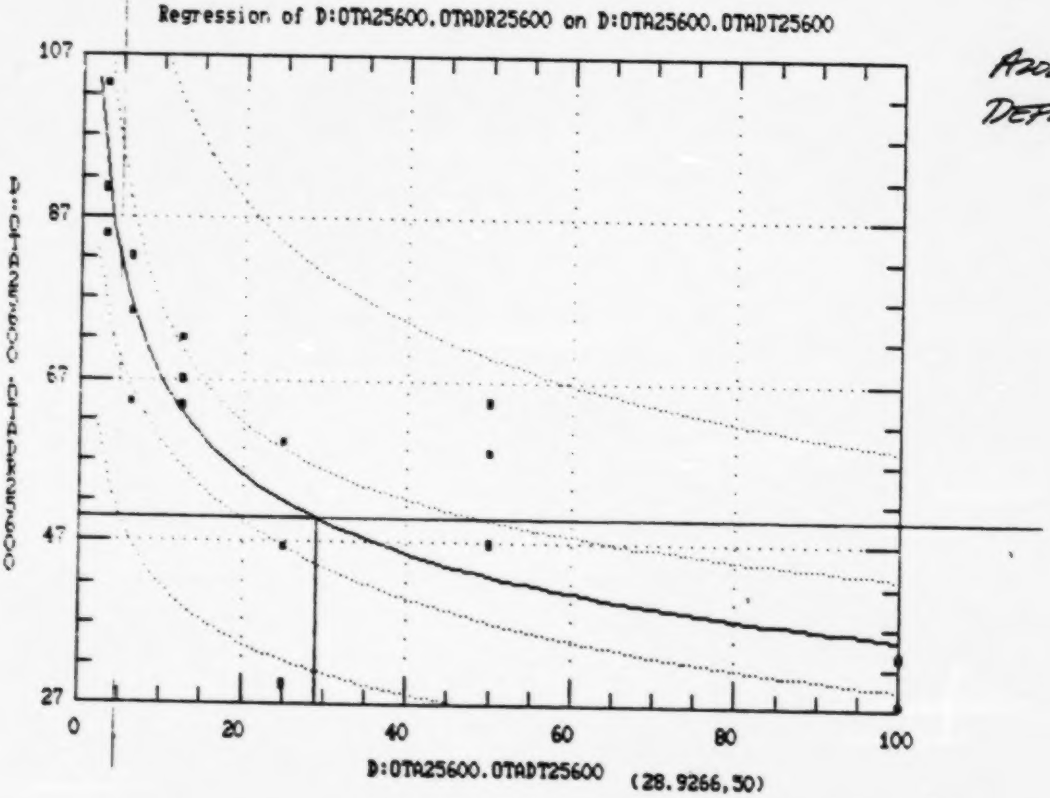


1 DROP A:OTA23600.OTURT21600

(0.147,50)

PHTHALLIC ANHYDRIDE
RANGE-FINDING TEST
EC_{50} = 0.15 mg/L
(N.C.I.)

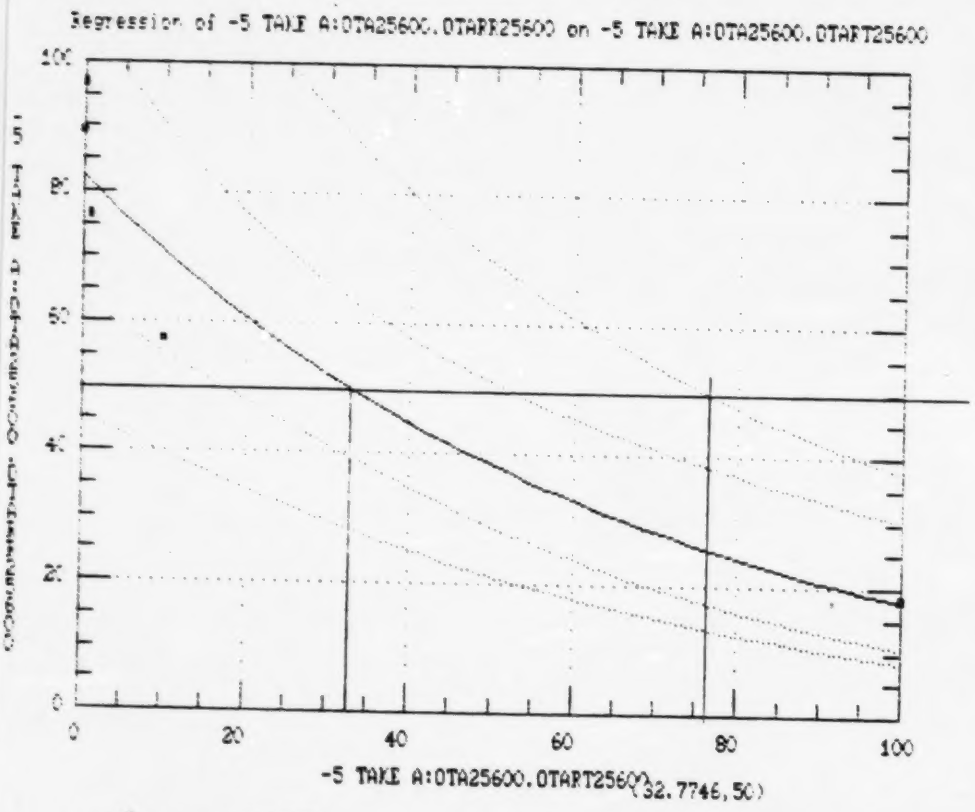
```
Regression Analysis - [redacted] odel: Y = aX^b
------------------------------------------------------------------------
Dependent variable: [redacted] ndependent variable: D:OTA25600.OTADT25
------------------------------------------------------------------------
                              Standard           T             Prob.
Parameter      Estimate        Error           Value           Level
------------------------------------------------------------------------
Intercept*      4.83465       0.134756         35.877              0
Slope          -0.274202      0.0433777       -6.32127       1.01634E-5
* NOTE: The Intercept is equal to Log a.
------------------------------------------------------------------------

                         Analysis of Variance
------------------------------------------------------------------------
Source               Sum of Squares    Df  Mean Square    F-Ratio  Prob. Level
Model                     1.897746      1     1.897746  39.958411       .00001
Error                      .759888     16      .047493
------------------------------------------------------------------------
Total (Corr.)             2.657634   [redacted]

Correlation Coefficient = -0.845029        [redacted]
Stnd. Error of Est. = 0.217929
```



AZOSULFHALIDE
DEFINITIVE

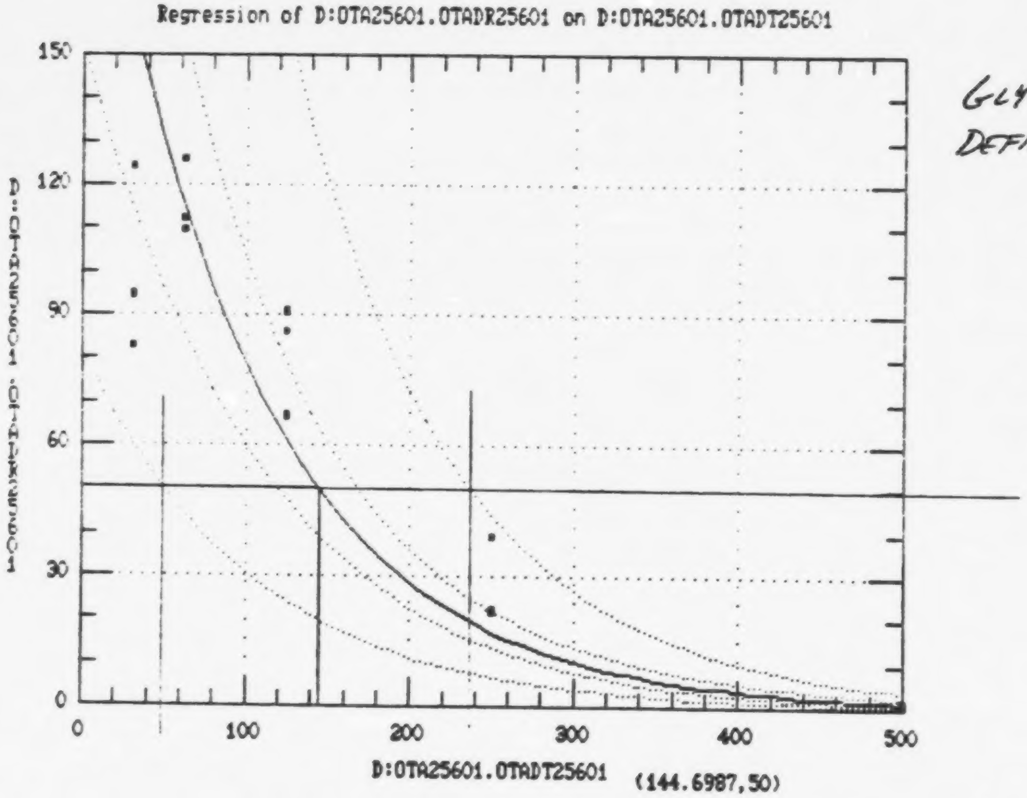
EC_{50} = 28.9 mg/L
(5-NC)

```
ression Analysis -[redacted]model: Y = exp(a+bX)
=========================================================================
endent variable: -5 TAKE A[redacted]Independent variable: -5 TAKE A:0TA25600
-------------------------------------------------------------------------
                           Standard             T               Prob.
ameter       Estimate        Error            Value             Level
-------------------------------------------------------------------------
ercept        4.41163      0.084485          52.2179        1.54682E-5
pe         -0.0152437    1.87967E-3         -8.10974        3.91899E-3
-------------------------------------------------------------------------

                      Analysis of Variance
-------------------------------------------------------------------------
rce             Sum of Squares     Df  Mean Square     F-Ratio  Prob. Level
el                   1.773422       1     1.773422   65.767887       .00392
or                   .0808946       3     .0269649
-------------------------------------------------------------------------
al (Corr.)          1.8543165    [redacted]

relation Coefficient = -0.977944          [redacted]
d. Error of Est. = 0.16421
```



AZOSULFAMIDE
RANGE-FINDING TEST
EC_{50} = 32.8 mg/L
(0-76)

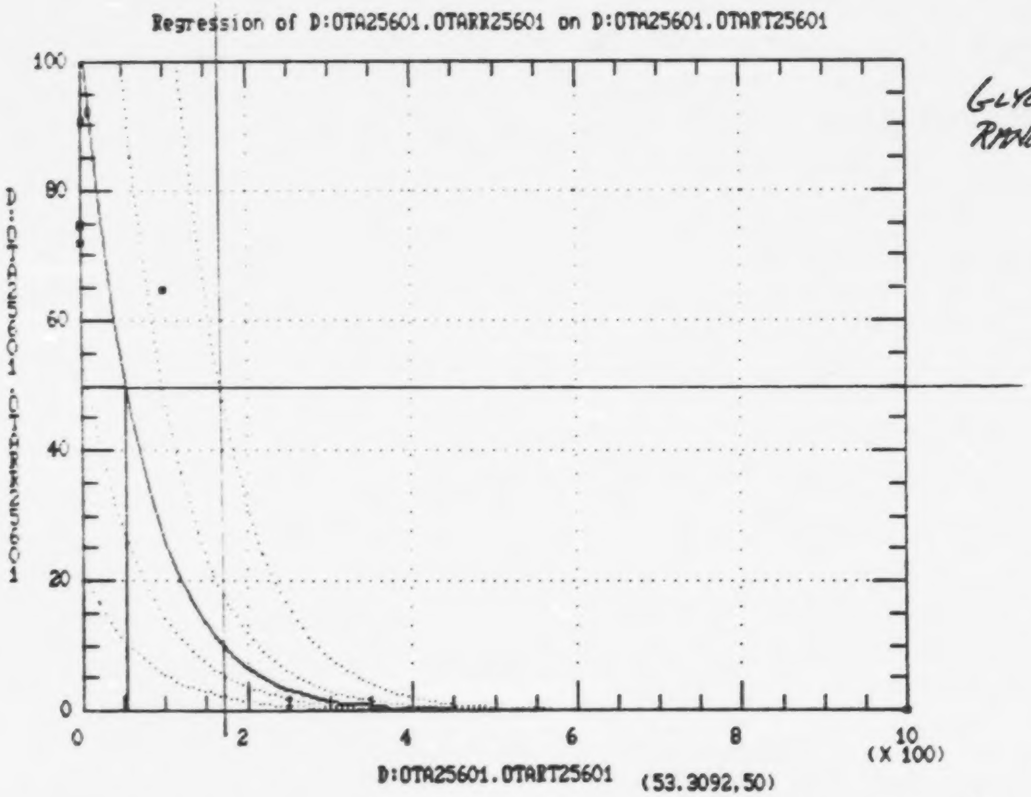
```
Regression Analysis - [redacted] odel: Y = exp(a+bX)
------------------------------------------------------------------------------
Dependent variable: [redacted] Independent variable: D:OTA25601.OTADT25
------------------------------------------------------------------------------
                            Standard           T              Prob.
Parameter     Estimate       Error            Value           Level
------------------------------------------------------------------------------
Intercept      5.37624      0.163577        32.8668      6.72795E-14
Slope       -0.0101191      6.3384E-4      -15.9647      6.38354E-10
------------------------------------------------------------------------------

                          Analysis of Variance
------------------------------------------------------------------------------
Source           Sum of Squares    Df  Mean Square    F-Ratio  Prob. Level
Model                  44.63819     1     44.63819  254.87235       .00000
Error                  2.276812    13      .175139
------------------------------------------------------------------------------
Total (Corr.)         46.915000  [redacted]

Correlation Coefficient = -0.975433       [redacted]
Stnd. Error of Est. = 0.418497
```



GLYCIDOL
DEFINITIVE

EC_{50} = 144.7 mg/L
(50 - 238)

```
Regression Analysis - [redacted] model: Y = exp(a+bX)
----------------------------------------------------------------------------
Dependent variable: [redacted] Independent variable: D:OTA25601.OTART25
----------------------------------------------------------------------------
                              Standard            T              Prob.
Parameter       Estimate        Error            Value            Level
----------------------------------------------------------------------------
Intercept        4.64588       0.238112         19.5113      4.06851E-5
Slope         -0.0137661      5.80329E-4       -23.7212      1.87274E-5
----------------------------------------------------------------------------

                         Analysis of Variance
----------------------------------------------------------------------------
Source              Sum of Squares    Df   Mean Square    F-Ratio   Prob. Level
Model                    152.42646     1     152.42646   562.69425       .00002
Error                    1.0835473     4      .2708868
----------------------------------------------------------------------------
Total (Corr.)            153.51001   [redacted]

Correlation Coefficient = -0.996465       [redacted]
Stnd. Error of Est. = 0.520468
```

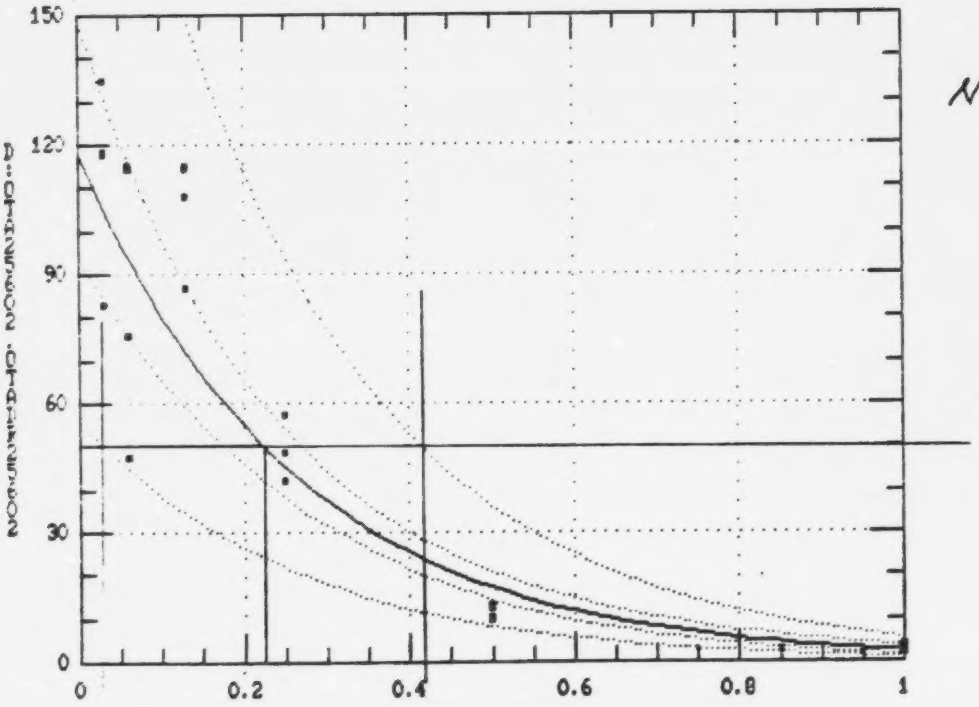


GLYCIDOL RANGE-FINDING

EC_{50} = 53.3 mg/L
(0 - 170)

```
Regression Analysis [redacted]model: Y = exp(a+bX)
------------------------------------------------------------------------
Dependent variable: [redacted]ndependent variable: D:OTA25602.OTADT25
------------------------------------------------------------------------
                                Standard          T            Prob.
Parameter        Estimate         Error          Value          Level
------------------------------------------------------------------------
Intercept         4.76897       0.109725        43.463              0
Slope            -3.82159       0.232712       -16.422    1.95024E-11
------------------------------------------------------------------------

                        Analysis of Variance
------------------------------------------------------------------------
Source               Sum of Squares    Df  Mean Square    F-Ratio  Prob. Level
Model                      30.10368     1     30.10368  269.68220       .00000
Error                      1.786024    16      .111627
------------------------------------------------------------------------
Total (Corr.)             31.889706 [redacted]

Correlation Coefficient = -0.971593     [redacted]
Stnd. Error of Est. = 0.334106
```

Regression of D:OTA25602.OTADR25602 on D:OTA25602.OTADT25602

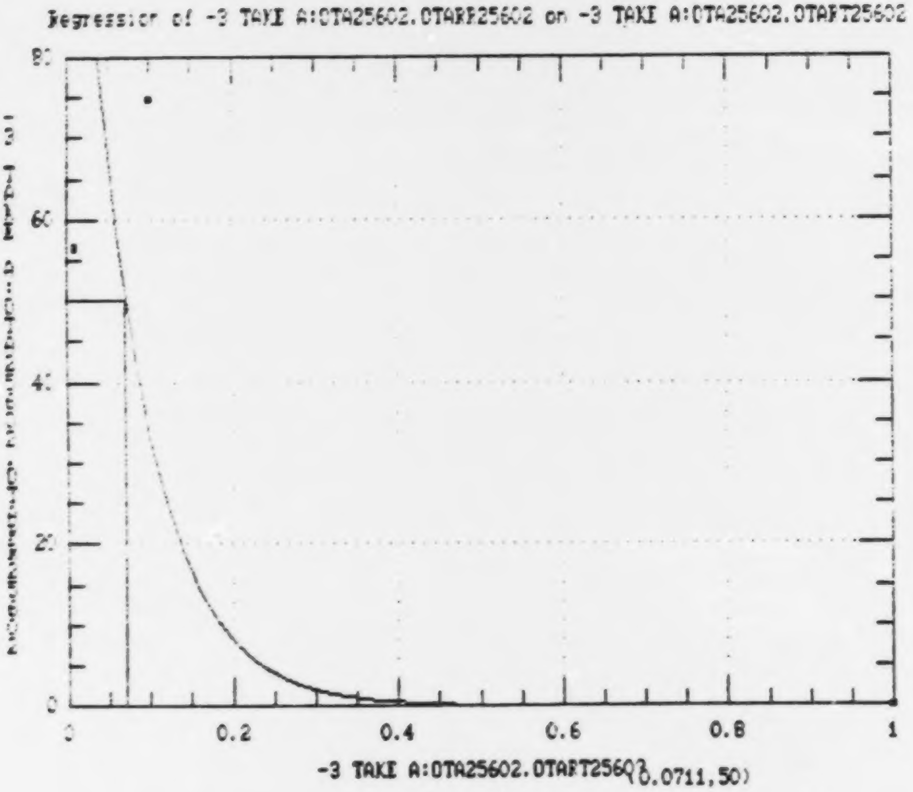


D:OTA25602.OTADT25602 (0.2242,50)

N-OCTYLAMINE
DEFINITIVE

EC_{50} = 0.22 mg/L
(.02-.42)

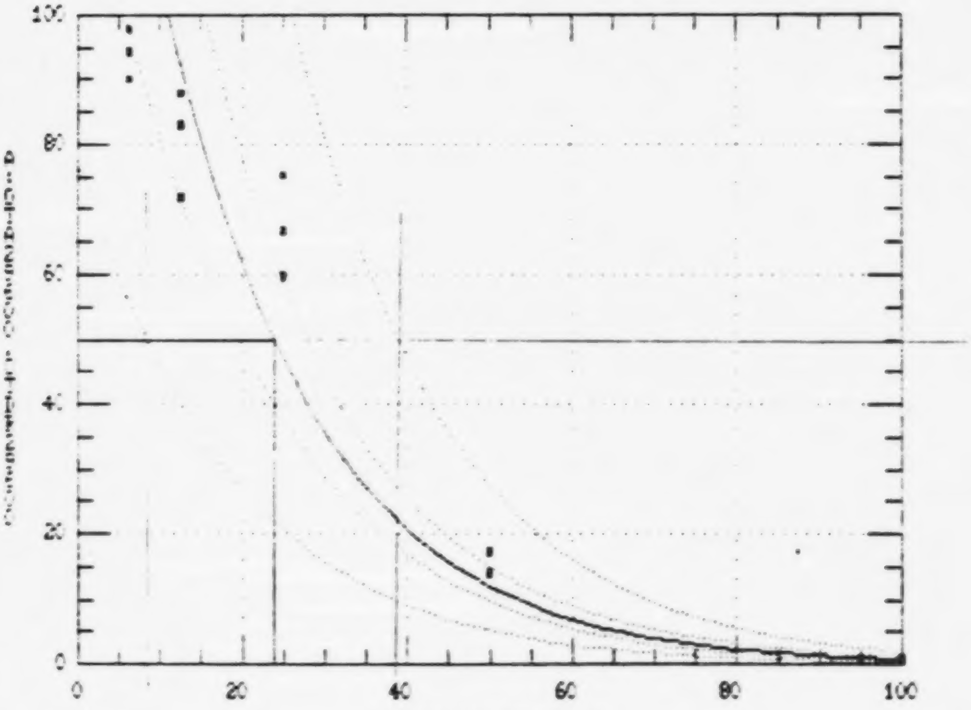
```
gression Analysis [redacted] odel: Y = exp(a+bX)
==============================================================================
pendent variable: -3 TAKE A[redacted]ndependent variable: -3 TAKE A:OTA25602
------------------------------------------------------------------------------
                         Standard              T              Prob.
rameter      Estimate      Error             Value             Level
------------------------------------------------------------------------------
tercept       4.91065     0.819451           5.9926          0.105264
ope          -14.0476      1.41222          -9.9472          0.0637856
------------------------------------------------------------------------------

                          Analysis of Variance
------------------------------------------------------------------------------
urce                Sum of Squares     Df  Mean Square     F-Ratio  Prob. Level
del                      118.28277      1    118.28277    98.94671        .06379
ror                      1.1954189      1    1.1954189
------------------------------------------------------------------------------
tal (Corr.)              119.47819   [redacted]

rrelation Coefficient = -0.994985          [redacted]
nd. Error of Est. = 1.09335
```



OCTYLAMINE
RANGE-FINDING TEST
EC_{50} = .07 mg/L
(NCI)

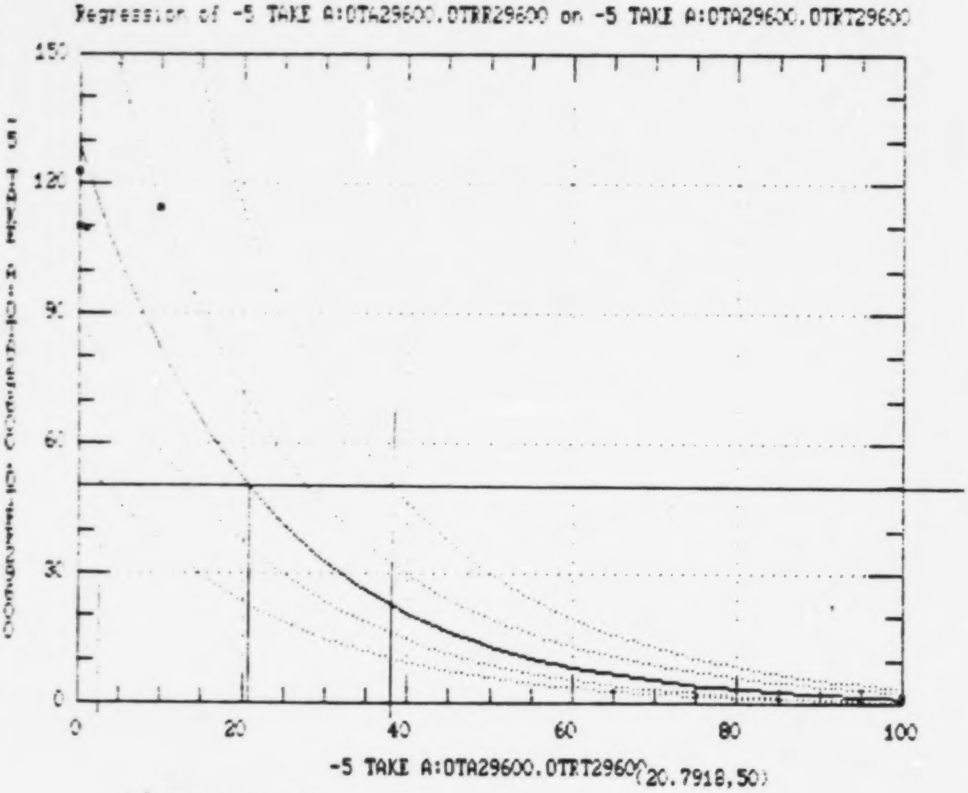
```
Regression Analysis - Exponential model: Y = exp(a+bX)
================================================================================
Dependent variable: A:OTA29600.OTDR29600Independent variable: A:OTA29600.OTDT296
--------------------------------------------------------------------------------
                                Standard              T                 Prob.
Parameter        Estimate        Error              Value              Level
--------------------------------------------------------------------------------
Intercept         5.19901       0.143295           36.2818        1.88738E-14
Slope           -0.054127       2.77625E-3        -19.4964        5.23872E-11
--------------------------------------------------------------------------------

                          Analysis of Variance
--------------------------------------------------------------------------------
Source                  Sum of Squares     Df  Mean Square    F-Ratio  Prob. Level
Model                         51.08725      1     51.08725  380.11088       .00000
Error                         1.747212     13      .134401
--------------------------------------------------------------------------------
Total (Corr.)                52.834457     14

Correlation Coefficient = -0.983326           R-squared =  96.69 percent
Stnd. Error of Est. = 0.366607
```

Regression of A:OTA29600.OTDR29600 on A:OTA29600.OTDT29600



NITROBENZENE
DEFINITIVE TEST -
FULL DATA
EC_{50} = 23.8 mg/L
(8-38)

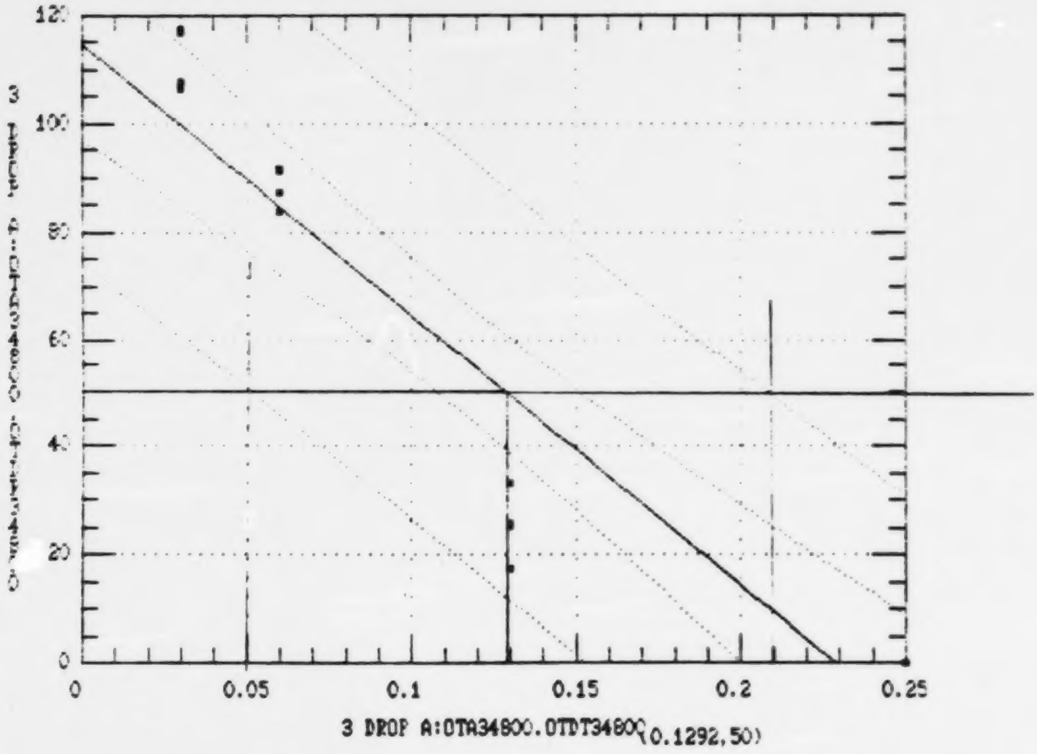
```
gression Analysis - [redacted] model: Y = exp(a+bX)
==========================================================================
pendent variable: -5 TAKE A[redacted].0Independent variable: -5 TAKE A:OTA29600
--------------------------------------------------------------------------
                            Standard            T              Prob.
rameter        Estimate       Error           Value            Level
--------------------------------------------------------------------------
tercept         4.85549     0.116199         41.7859        3.01639E-5
ope          -0.0453765    2.58527E-3        -17.552        4.03126E-4
--------------------------------------------------------------------------

                         Analysis of Variance
--------------------------------------------------------------------------
urce                 Sum of Squares    Df  Mean Square    F-Ratio  Prob. Level
del                        15.71436     1     15.71436  308.07180        .00040
ror                        .1530262     3     .0510087
--------------------------------------------------------------------------
tal (Corr.)               15.867383    [redacted]

rrelation Coefficient = -0.995166          [redacted]
d. Error of Est. = 0.225851
```



NITROBENZENE
RANGE-FINDING TEST
EC_{50} = 20.8 mg/L
(3-38)

```
Regression Analysis - Linear model: Y = a+bX
==============================================================================
Dependent variable: 3 DROP A:0TA34800.0TIndependent variable: 3 DROP A:0TA34800.
------------------------------------------------------------------------------
                              Standard            T              Prob.
Parameter       Estimate        Error            Value            Level
------------------------------------------------------------------------------
Intercept        114.752       8.07886          14.204       5.89762E-8
Slope           -501.321       55.7826        -8.98705       4.19194E-6
------------------------------------------------------------------------------

                          Analysis of Variance
------------------------------------------------------------------------------
Source                  Sum of Squares     Df  Mean Square    F-Ratio  Prob. Level
Model                        21620.074      1    21620.074     80.767       .00000
Error                        2676.8398     10     267.6840
------------------------------------------------------------------------------
Total (Corr.)                24296.914     11

Correlation Coefficient = -0.943307          R-squared =  88.98 percent
Stnd. Error of Est. = 16.3611
```

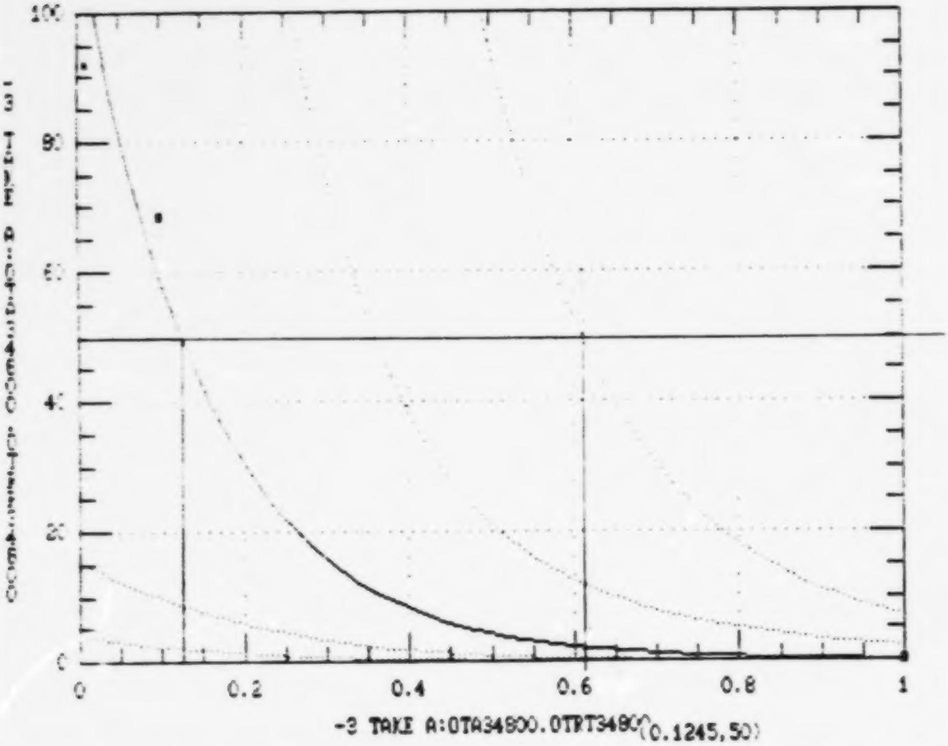
Regression of 3 DROP A:0TA34800.0TDR34800 on 3 DROP A:0TA34800.0TDT34800



METHYL VINYL KETONE
DEFINITIVE TEST
DROPPED HIGH CONC.
EC_{50} = .13 mg/L
(.05-.21)

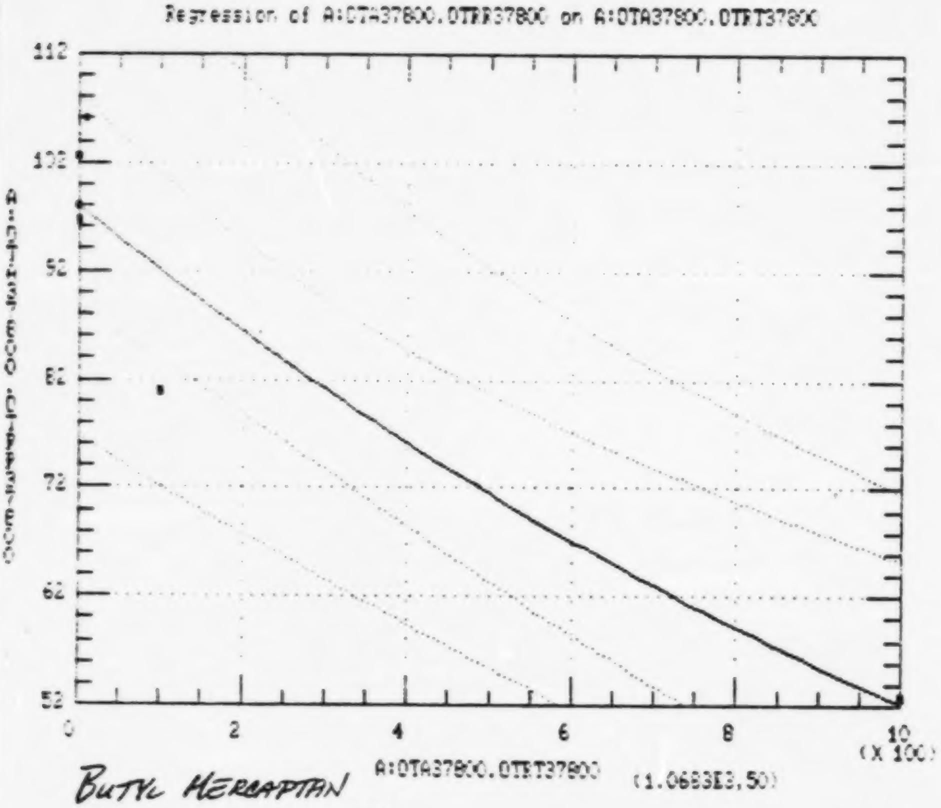
```
ression Analysis [redacted]:del: Y = exp(a+bX)
===========================================================================
endent variable: -3 TAKE A[redacted]Independent variable: -3 TAKE A:OTA34800
---------------------------------------------------------------------------
                           Standard              T               Prob.
ameter         Estimate        Error             Value            Level
---------------------------------------------------------------------------
ercept          4.72704     0.156842           30.1389         0.0211151
pe             -6.54558     0.270297          -24.2163          0.026274
---------------------------------------------------------------------------

                        Analysis of Variance
---------------------------------------------------------------------------
rce                  Sum of Squares    Df  Mean Square    F-Ratio  Prob. Level
del                        25.68105     1     25.68105  586.42783       .02627
ror                        .0437924     1     .0437924
---------------------------------------------------------------------------
tal (Corr.)               25.724846   [redacted]

rrelation Coefficient = -0.999148          [redacted]
nd. Error of Est. = 0.209266
```

Regression of -3 TAKE A:OTA34800.OTRR34800 on -3 TAKE A:OTA34800.OTRT34800



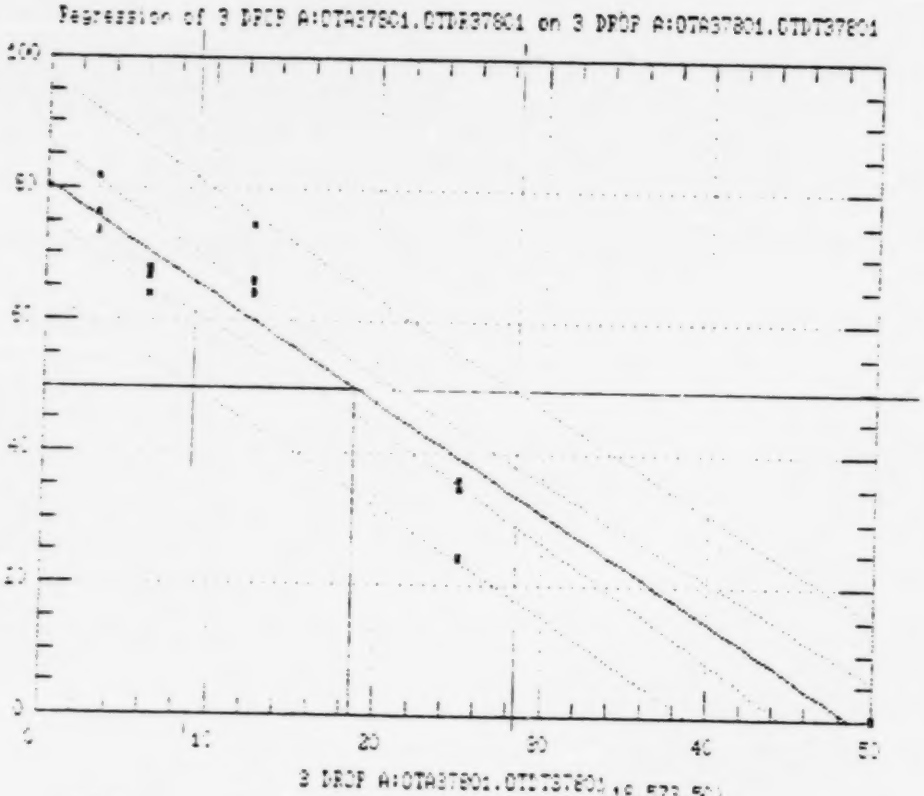
METHYL VINYL KETONE
RANGE-FINDING TEST
EC_{50} = .12 mg/L
(0-.61)

```
gression Analysis - [redacted] model: Y = exp(a+bX)
================================================================================
pendent variable: A:[redacted]TRR37800Independent variable: A:OTA37800.OTRT378
--------------------------------------------------------------------------------
                          Standard              T                 Prob.
rameter       Estimate       Error            Value               Level
--------------------------------------------------------------------------------
tercept        4.58552      0.036987         123.977          2.53865E-8
ope        -6.30458E-4     9.01451E-5        -6.99362           2.1994E-3
--------------------------------------------------------------------------------

                         Analysis of Variance
--------------------------------------------------------------------------------
urce                 Sum of Squares     Df   Mean Square    F-Ratio  Prob. Level
del                        .319707       1      .319707   48.913489       .00220
ror                       .0261447       4     .0065362
--------------------------------------------------------------------------------
tal (Corr.)               .3458518   [redacted]

rrelation Coefficient = -0.96146           [redacted]
nd. Error of Est. = 0.0808466
```



BUTYL MERCAPTAN
RANGE-FINDING TEST
EC_{50} = 1068 mg/L
(NCI)

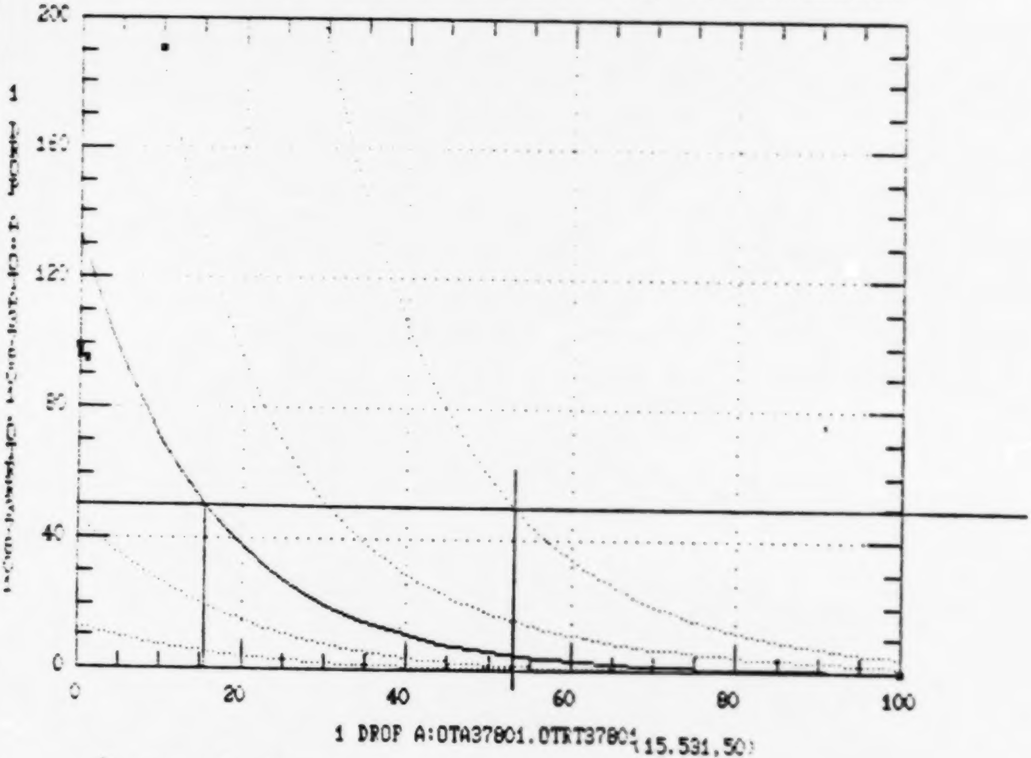
```
ression Analysis - Linear model: Y = a+bX
==========================================================================
endent variable: 3 DROP A:OTA37801.OTIndependent variable: 3 DROP A:OTA37801.
--------------------------------------------------------------------------
                          Standard           T              Prob.
ameter        Estimate      Error          Value            Level
--------------------------------------------------------------------------
ercept         80.7765      2.75626        29.3065     2.93099E-13
pe            -1.65706     0.106801       -15.5154     9.09392E-10
--------------------------------------------------------------------------

                          Analysis of Variance
--------------------------------------------------------------------------
rce                 Sum of Squares    Df  Mean Square     F-Ratio  Prob. Level
el                       11968.839     1    11968.839     240.726       .00000
or                       646.35634    13     49.71972
--------------------------------------------------------------------------
al (Corr.)               12615.196    14

relation Coefficient = -0.974045           R-squared =  94.88 percent
d. Error of Est. = 7.05122
```



METHYL ACRYLATE
DEFINITIVE TEST
EC_{50} = 18.6 mg/L
(9 - 28)

```
gression Analysis - [redacted]  model: Y = exp(a+bX)
================================================================================
sendent variable: 1 DROP A:[redacted]OTIndependent variable: 1 DROP A:OTA37801.
--------------------------------------------------------------------------------
                           Standard              T                 Prob.
rameter       Estimate       Error             Value               Level
--------------------------------------------------------------------------------
tercept        4.89209      0.332781          14.7006          6.82769E-4
ope         -0.0631039     7.4039E-3         -8.52306          3.39289E-3
--------------------------------------------------------------------------------

                        Analysis of Variance
--------------------------------------------------------------------------------
urce             Sum of Squares     Df  Mean Square    F-Ratio   Prob. Level
del                   30.391136      1    30.391136  72.642582        .00339
ror                   1.2550959      3     .4183653
--------------------------------------------------------------------------------
tal (Corr.)           31.646232     [redacted]

rrelation Coefficient = -0.979969              [redacted]
nd. Error of Est. = 0.646812
```

Regression of 1 DROP A:OTA37801.OTRR37801 on 1 DROP A:OTA37801.OTRT37801



METHYL ACRYLATE
RANGE-FINDING TEST
HIGH CONC. DROPPED
EC_{50} = 15.5 mg/L
(0-53)

APPENDIX C

RAW DATA

TEST CODE: OT040389 A

TEST WATER: Glyoxal

CHEM NO. OTP 14600

DATE SAMPLED: —

DATE ELUTED: —

	high conc.	low conc.
INITIAL pH:	9.3	7.2
FINAL pH:	4.98	7.1

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 03

INOCULUM:

Selenastrum ALGA

~10^4 CELLS/ML

0.1591 MG/L DRY WT.

44.2 MCV

0.37 MLS INOCULUM

TEST PREPARATION:

TEST WATERS: NB AAM BATCH ~~[illegible]~~ MEN12800

DILUTION MEDIA: NB

INOCULUM: NB

INOCULATED: NB

SAMPLED: NB

COUNTED: NB

TEST/CONTAINER VOLUME: 50/125

REPLICATE FLASKS: 3

DILUTION RANGE: ~~[illegible]~~ 1000 mg/L – 62.5 mg/L

NOTES:

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE DT040389A CHEM. NUMBER OTP 14600
SHAKER NUMBER 211 TEST MATERIAL glyoxal

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	040389A / HB	3:00pm	100	410-440
DAY 1	040489A / HB	3:15pm	104	410-440
DAY 2	040589/HB	2:35pm	104	410-440
day 3	040689/HB	3:05pm	104	400-440
DAY 4	040789/HB	2:00pm	100	400-440
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

TEST WATER: Controls for: glyoxal TEST CODE: ~~[illegible]~~ OT040389A

TREATMENT: SPECIES: Selenastrum

CHEM. #: OTP14600 SPIKE: AAM NEG. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
04	20	196	1	2656	50	2675	51	2771	51
			2	1555	78	1143	79	1137	81
			3	708	68	741	72	726	75
	20	107	1	18181	47	18253	43	17988	46
			2	4019	55	3941	53	4120	53
			3	3620	55	3628	54	3686	54
	200	460	1	11490	36	10987	35	10143	36
			2	1452	48	1271	50	1309	50
			3	1548	50	1668	51	1509	52
	200	332	1	22495	29	26628	30	26699	29
			2	5448	68	15585	67	5796	66
			3	5979	68	6091	69	6395	70
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: Zn POS. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV	
04	20	196	1	402	56	425	48	464	55	.48 mg/L
			2	691	62	699	69	741	60	.74 mg/L
			3	832	65	792	64	792	62	.38 mg/L
	20	107	1	617	61	657	62	675	59	
			2	2176	52	2359	57	2270	57	
			3	1246	62	1301	61	1389	61	
	20	460	1	876	71	725	71	870	68	
			2	2694	74	2794	70	2883	67	
			3	1565	64	1558	64	1672	62	
	20	332	1	2438	56	2515	58	2511	66	
			2	4249	66	4120	63	4433	67	
			3	2818	55	2804	56	2835	56	
			1							
			2							
			3							
			1							
			2							
			3							

TEST WATER: glyoxal

TEST CODE: OT040389A

TREATMENT:

SPECIES: Selenastrum

CHEM. #: OTP14600

SPIKE: 1000 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
	26	106	1	578	51	569	44	645	44
			2	560	41	581	44	573	45
			3	680	40	671	42	651	47
	29	167	1	636	41	693	44	741	44
			2	557	44	575	40	630	42
			3	561	45	682	41	661	43
	20	4610	1	1111	50	1036	50	1134	52
			2	1112	61	1064	59	1069	64
			3	925	47	875	46	967	46
	20	332	1	1857	41	1826	45	1926	42
			2	1756	41	1720	42	1988	46
			3	1871	39	1831	41	1933	41
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 500 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
	26	106	1	597	41	642	44	613	45
			2	594	45	619	45	674	44
			3	653	54	630	59	617	57
	50	107	1	705	45	743	45	735	51
			2	606	45	624	47	574	43
			3	1036	52	978	47	1002	50
	20	4610	1	824	48	829	47	864	50
			2	761	39	758	42	757	37
			3	1450	52	1371	51	1441	53
	20	332	1	1924	46	1799	45	1923	48
			2	1933	44	1772	42	1805	45
			3	2672	45	2682	48	2571	48
			1						
			2						
			3						
			1						
			2						
			3						

5.5

TEST WATER: glyoxal

TEST CODE: OT040389A

TREATMENT:

SPECIES: Selenastrum

CHEM. #: OTP14600

SPIKE: 250 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
	20	106	1	1220	55	1227	55	1316	53
			2	891	66	909	63	917	59
			3	716	67	687	62	690	61
	20	107	1	7101	44	7110	44	6943	45
			2	3938	67	3795	66	3865	65
			3	3108	69	3128	65	3253	66
	20	466	1	27576	39	27340	38	27236	38
			2	12699	59	13606	58	13616	59
			3	13517	61	14137	61	14174	61
1	200	332	1	7643	35	7855	35	7325	35
			2	4762	48	4698	49	4863	48
			3	4969	51	4955	51	4927	49
			1						
			2						
			3						
			1						
			2						
			3						

pH
6.87

SPIKE: 125 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
	20	106	1	2240	50	2184	49	2284	47
			2	696	78	672	84	777	72
			3	1186	63	1300	59	1267	63
	20	107	1	10410	49	10226	49	10284	49
			2	1182	82	1119	84	1111	78
			3	4398	60	4431	59	4759	59
	20	460	1	38003	49	35605	49	40526	49
			2	4636	57	4773	55	4861	58
			3	11823	58	12602	59	13125	60
	200	332	1	11806	40	12280	40	12633	39
			2	1729	51	1831	51	1930	49
			3	4054	50	4167	49	4134	51
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: glyoxal

TEST CODE: OT 04 03 89A

TREATMENT:

SPECIES: Selenastrum

CHEM. #: OTP14600

SPIKE: 62.5 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
	20	106	1	759	70	767	68	815	74
			2	2345	46	2356	46	2308	44
			3	1687	52	1701	50	1766	49
	50	107	1	2225	72	2366	74	2387	74
			2	11317	56	11093	56	11281	55
			3	7017	51	6928	50	7070	53
	50	460	1	4002	73	4153	73	4195	74
			2	52701	44	53224	44	53282	44
			3	16875	51	17283	52	17765	52
	500	332	1	4726	47	14780	47	14924	45
			2	16539	36	16360	35	16715	36
			3	5832	44	5844	46	5820	45
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE:

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST CODE: OT022889

TEST WATER: GLYOXAL*

CHEM NO. OTH0960D

DATE SAMPLED: —

DATE ELUTED: —

TIME ON: 2:20p
ct. day 1: 3:00p
day 2 2:45
day 3 2:15
day 4 2:40

	high conc.	low conc.
INITIAL pH:	4.5	7.3
FINAL pH:	4.4	9.3

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 5

INOCULUM:

Selenastrum ALGA
10,000 CELLS/ML
.1706 MG/L DRY WT.
47.4 MCV
.53 MLS INOCULUM

TEST PREPARATION:

TEST WATERS: MB AAM BATCH MEN08800

DILUTION MEDIA: MB

INOCULUM: MB

INOCULATED: MB

SAMPLED: HB

COUNTED: HB

TEST/CONTAINER VOLUME: 50

REPLICATE FLASKS: 1

DILUTION RANGE: 1000 - .01 mg/L

NOTES: *RANGE-FINDING TEST ; SPECIAL O.T.S. PROCEDURE (SEE FILES) DAILY COUNT; Zn^{++} POSITIVE CONTROL WAS 37, 74, & 148 µg/L 1X INSTEAD OF 74 µg/L 3X

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE OT022889 CHEM. NUMBER OTN09600

SHAKER NUMBER 208 TEST MATERIAL GYOXAL

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	022889 / NB	2:20 pm	108	NO METER
DAY 1	030189/NB	2:50pm	108	390 - 410 FC
DAY 2	030289/NB	2:45pm	108	380-410
day 3	030389/NB	2:15pm	108	380-400
DAY 4	030489/NB	2:40pm	108	380-400
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

TEST WATER: Controls for: GLYOXAL TEST CODE: 01 022887

TREATMENT: RANGE FINDING TEST SPECIES: Selenastrum

CHEM. #: MEN08800 SPIKE: AAM NEG. CONTROL

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	117	1	1828	42	1872	40	1827	42
			2	2211	41	2216	44	2193	41
			3	1739	39	1671	36	1758	37
02	20	139	1	6493	48	7268	48	7655	49
			2	11713	57	11638	57	11633	57
			3	13851	56	13542	55	13714	55
03	200	82	1	4462	49	4485	50	5031	51
			2	10385	50	9925	47	9978	48
			3	10320	39	10487	39	10664	38
04	200	84	1	13638	46	13594	46	14083	45
			2	20258	45	23247	45	23520	45
			3	22871	43	23895	42	24115	43
			1						
			2						
			3						
			1						
			2						
			3						

CHEM #: 6319600 SPIKE: Zn POS. CONTROL

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV	
01	20	117	1	617	50	633	50	658	47	148 mg/L
			2	1706	46	1716	44	1686	47	74 "
			3	760	56	716	53	711	56	37 "
02	20	139	1	702	67	678	60	632	71	
			2	3353	69	3693	68	3750	69	
			3	1052	55	1100	53	1129	57	
03	20	82	1	807	69	729	86	724	84	
			2	13500	51	13763	51	14455	51	
			3	1279	62	1224	62	1200	61	
04	20	84	1	800	85	782	70	795	75	
			2	35049	52	35966	50	36075	51	
			3	1613	61	1443	61	1683	66	
			1							
			2							
			3							
			1							
			2							
			3							

TEST WATER: GLYOXAL TEST CODE: OT022889

TREATMENT: RANGE-FINDING TEST SPECIES: Selenastrum

CHEM. #: OTN096OO SPIKE: 1000 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	117	1	576	35	530	35	564	38
			2						
			3						
02	20	139	1	519	46	541	44	491	39
			2						
			3						
03	20	82	1	541	35	542	43	526	37
			2						
			3						
04	20	84	1	1402	29	1433	28	1393	27
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 100 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	117	1	2074	42	2065	39	2080	41
			2						
			3						
02	20	139	1	8778	58	8747	58	8677	59
			2						
			3						
03	200	82	1	5405	46	5699	46	5789	46
			2						
			3						
04	200	84	1	18148	36	18943	37	19056	37
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TREATMENT: RANGE-FINDING TEST SPECIES: Selenastrum

CHEM. #: OTN096CC SPIKE: 10mg/L

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	117	1	2086	44	2180	40	2215	42
			2						
			3						
02	1 20	139	1	13079	53	13414	52	13552	53
			2						
			3						
03	200	82	1	8338	39	8968	40	8929	39
			2						
			3						
04	200	84	1	22671	37	23201	37	23550	37
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 1 mg/L

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	117	1	1972	42	2000	40	2078	43
			2						
			3						
02	1 20	139	1	14662	49	15457	48	15242	48
			2						
			3						
03	200	82	1	9332	36	9464	36	9532	37
			2						
			3						
04	200	84	1	24493	36	24391	35	25757	35
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TREATMENT: RANGE-FINDING TEST

SPECIES: Selenastrum

CHEM. #: ~~45~~ OTN09600

SPIKE: .1 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	117	1	1613	40	1704	41	1784	38
			2						
			3						
02	20	139	1	6329	43	6226	44	6362	44
			2						
			3						
03	200	82	1	1448	46	1508	46	1416	45
			2						
			3						
04	200	84	1	2284	52	2384	49	2736	49
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: .01 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	117	1	2035	42	1983	44	2043	41
			2						
			3						
02	20	139	1	12655	49	12770	49	12765	49
			2						
			3						
03	200	82	1	8778	38	8843		8788	37
			2						
			3						
04	200	84	1	35405	36	30276	35	35641	35
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

0T050889
S.D.B.S.

TEST CODE: Na-dodecylbenzene sufonic acid

TEST WATER: O.T.S.

CHEM NO. S.D.B.S.

DATE SAMPLED: —

DATE ELUTED: —

	high conc.	low conc.
INITIAL pH:	7.5	7.5
FINAL pH:	7.6	8.8

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 04

INOCULUM:

$\sim 10^4$ ALGA
Selenastrum c. CELLS/ML
0.1159 MG/L DRY WT.
32.2 MCV
0.44 MLS INOCULUM

TEST PREPARATION:

TEST WATERS: HB AAM BATCH 15800

DILUTION MEDIA: HB

INOCULUM: HB

INOCULATED: HB

SAMPLED: HB

COUNTED: HB

TEST/CONTAINER VOLUME: 50

REPLICATE FLASKS: 3

DILUTION RANGE: 250 mg/L - 15.625 mg/L

NOTES: O.T.S.

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE SC050889 CHEM. NUMBER

SHAKER NUMBER 204 TEST MATERIAL Na-dodecylbenzene sulfonate

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	050889/JHB	11:10 AM	100	360-410
DAY 1	050989/JHB	10:45 AM	100	360-410
DAY 2	051089/JHB	11:00 A.M.	100	360-410
day 3	051189/JHB	11:00 A.M.	100	360-410
DAY 4	051289/JHB	10:30 A.M	100	360-400
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

TEST WATER: Controls for:

TEST CODE: ~~DC~~

TREATMENT:

SPECIES: Selenastrum

S.D.B.S.

Na-dodecylbenzene sulfonate

CHEM. #:

SPIKE: AAM NEG. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	182	1	11396	35	11426	40	11436	39
			2	11336	34	11405	38	11417	37
			3	11317	33	11351	36	11487	35
02	120	59	1	5419	45	5500	46	5559	45
			2	4946	46	5246	46	5071	46
			3	5171	42	5213	42	5041	42
03	200	120	1	3999	46	4544	48	4301	47
			2	3160	39	3043	37	2045	38
			3	3129	39	3071	37	3188	36
04	200	126	1	11312	47	11234	47	11181	46
			2	10674	43	113[illegible]	43	11562	42
			3	10977	39	10758	40	11718	40
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: Zn POS. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV	
01	120	182	1	347	42	366	42	349	46	1.48
			2	620	43	606	38	642	41	.74
			3	836	45	848	44	855	43	.38
02	120	59	1	508	89	583	80	545	87	
			2	1862	52	1827	51	1873	52	
			3	1462	52	1363	49	1355	51	
03	120	120	1	575	65	576	64	538	65	
			2	1520	48	1477	48	1514	46	
			3	4598	50	4699	51	4783	49	
04	120	126	1	609	64	758	60	703	55	
			2	2339	56	2387	55	2514	53	
			3	13380	55	13397	51	13411	55	
			1							
			2							
			3							
			1							
			2							
			3							

TEST WATER: OTS

TEST CODE:

TREATMENT:

SPECIES: Selenastrum

CHEM. #: (6)

SPIKE: 250 mg/L SDBS

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	182	1	1266	57	1225	54	1380	47
			2	1092	44	1161	48	1186	43
			3	1022	54	1113	50	1090	48
02	1 20	59	1	1889	54	1791	50	1866	51
			2	2161	48	1830	49	1800	46
			3	1628	52	1654	47	1666	50
03	1 20	1/210	1	2743	57	2820	59	2787	52
			2	2860	62	2633	56	2766	56
			3	2506	58	2384	58	2392	59
04	1 20	/526	1	4249	62	4370	66	4428	65
			2	4032	63	3962	61	4127	60
			3	3313	60	3428	60	3549	62
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 125 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	182	1	845	54	826	54	855	56
			2	959	55	918	57	936	62
			3	865	59	877	62	933	57
02	1 20	59	1	1285	86	1141	74	1053	70
			2	1130	74	1070	73	1206	56
			3	1450	79	1291	71	1309	72
03	1 210	/120	1	1565	91	1580	80	1732	80
			2	1798	84	1918	82	1947	81
			3	2623	86	2820	87	2886	86
04	1 20	/526	1	[illegible]432	80	3785	77	4145	73
			2	4717	79	4733	77	5074	76
			3	7285	87	7486	87	7567	86
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: U12

TEST CODE: OT050884

TREATMENT:

SPECIES: Selenastrum

CHEM. #: 63

SPIKE: 62.5 mg/L S.D.B.S.

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	812	1	979	61	963	54	1035	51
			2	1046	58	1111	59	1063	52
			3	816	77	779	69	818	63
02	120	159	1	3820	65	3752	67	3835	70
			2	3428	74	3381	70	3418	67
			3	2483	72	3504	71	2450	73
03	120	1120	1	13820	72	14807	72	14889	72
			2	14231	74	14524	73	14583	75
			3	9122	78	9911	77	10950	77
04	120	1126	1	34216	82	37350	81	38276	81
			2	31972	81	34109	81	33220	81
			3	29750	81	30621	82	30567	79
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 31.25 mg/L

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	812	1	1183	56	1219	51	1179	57
			2	929	58	869	56	927	59
			3	1052	49	1048	50	1053	50
02	120	159	1	4061	63	4051	61	4101	61
			2	4329	64	4240	64	4216	59
			3	4669	62	4587	58	4504	57
03	120	1120	1	16531	65	16974	65	18138	65
			2	16902	64	18138	64	20137	63
			3	19771	59	20167	60	22704	59
04	200	1156	1	4626	66	4414	66	5007	68
			2	5114	68	5168	70	6122	71
			3	6779	65	6724	64	6731	67
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: OTS

TEST CODE: OT 050807

TREATMENT:

SPECIES: [illegible]

CHEM. #: 63

SPIKE: 15.625 mg/L S.DBS

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	182	1	11110	50	11056	49	11181	45
			2	11239	51	11259	49	11280	45
			3	11131	49	11162	44	11147	46
02	1 20	59	1	4827	58	5093	57	5130	57
			2	5367	58	5566	55	5310	56
			3	5071	57	5367	56	5369	56
03	20	1520	1	24981	57	25497	57	25472	58
			2	27576	58	29176	57	28123	57
			3	23347	61	24569	60	26847	59
04	1200	1526	1	16098	64	16234	66	16228	65
			2	8059	60	7978	61	8312	57
			3	8031	57	8098	58	8048	57
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE:

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST CODE: OTD50189

TEST WATER: S.D.S. SODIUM DODECYLBENZENE SULFONIC ACID.

CHEM NO. —

DATE SAMPLED: —

DATE ELUTED: —

ON @ 3:15

	high conc.	low conc.
INITIAL pH:	7.5	7.5
FINAL pH:	7.7	8.7

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 4

INOCULUM:

Selenastrum	ALGA
10^4	CELLS/ML
.1257	MG/L DRY WT.
34.6	MCV
.37	MLS INOCULUM

TEST PREPARATION:

TEST WATERS: MB AAM BATCH MEN15800

DILUTION MEDIA: MB

INOCULUM: MB

INOCULATED: MB

SAMPLED: HB

COUNTED: HB

TEST/CONTAINER VOLUME: 50/125

REPLICATE FLASKS: 1

DILUTION RANGE: 1000 - .01 mg/L

NOTES: * RANGE-FINDING TEST; SPECIAL O.T.S PROCEDURE, DAILY COUNT, NEW ZINC # CONTROL

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE 0T050189 CHEM. NUMBER N/A

SHAKER NUMBER 209 TEST MATERIAL S.D.S.

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	050189 -MB	3:25 P	104	340-400
DAY 1	050289 HB	3:30pm	106	360-400
DAY 2	050389 HB	3:15pm	104	360-400
day 3	050489 HB	3:15	106	340-390
DAY 4	050589 HB	3:30	104	340-390
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

TEST WATER: OT050189
TEST CODE:

TREATMENT: S.D.S
SPECIES: Selenastrum

CHEM. #: N/A

SPIKE: 1000 mg/L

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	156	1	633	49	672	51	754	51
			2						
			3						
02	120	110	1	877	55	871	51	937	53
			2						
			3						
03	120	115	1	810	47	788	47	827	44
			2						
			3						
04	120	95	1	1485	52	13016	57	11279	55
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 100 mg/L

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	156	1	819	61	877	56	895	55
			2						
			3						
02	120	110	1	3038	73	3057	74	3125	73
			2						
			3						
03	120	115	1	8842	82	8814	82	8905	80
			2						
			3						
04	120	95	1	24345	83	24301	83	24801	82
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: S.D.S

TEST CODE: 0T050189

TREATMENT:

SPECIES: Selenastrum

CHEM. #: N/A

SPIKE: 0 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	156	1	11348	42	11350	41	11459	42
			2						
			3						
02	120	110	1	7051	48	7113	52	7134	49
			2						
			3						
03	120	115	1	31389	55	33412	55	33065	55
			2						
			3						
04	200	75	1	8439	56	8319	56	8215	53
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 1 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	156	1	866	41	809	41	920	42
			2						
			3						
02	120	110	1	4629	41	4695	42	4608	41
			2						
			3						
03	120	115	1	24854	56	24965	56	25291	56
			2						
			3						
04	200	75	1	7555	49	7818	49	7871	52
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: SDS

TEST CODE: 0T 050189

TREATMENT: RANGE - FINDING

SPECIES: [illegible]

CHEM. #: N/A

SPIKE: .1

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	156	1	670	34	693	35	716	37
			2						
			3						
02	20	110	1	3839	47	4068	48	4110	47
			2						
			3						
03	20	115	1	29461	47	29986	47	30278	47
			2						
			3						
04	200	195	1	18274	44	19267	43	19756	43
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: .01

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	156	1	1054	34	1026	35	1096	33
			2						
			3						
02	20	110	1	4469	48	4464	50	4521	49
			2						
			3						
03	20	115	1	33197	43	33547	43	33225	43
			2						
			3						
	200	195	1	10443	35	10557	35	11409	35
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: Controls for: 05-01-89 TEST CODE: [redacted] 8T050189

TREATMENT: S.D.S SPECIES: Selenastrum

CHEM. #:

SPIKE: AAM NEG. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	156	1	1056	34	1030	32	1044	35
			2	1097	34	1151	33	1066	35
			3	951	36	1066	35	969	37
02	20	110	1	4084	48	4263	48	4296	48
			2	4197	47	4504	47	4421	46
			3	3893	49	3890	49	3965	50
03	20	115	1	29829	43	30014	43	29329	42
			2	32878	41	32771	42	32452	42
			3	28907	41	27111	42	30207	41
04	200	95	1	10526	34	11193	34	11122	33
			2	11021	31	11409	30	12289	35
			3	11059	32	13540	30	15591	30
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: Zn POS. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV	
01	50	151	1	349	45	358	44	350	47	128
			2	1638	37	705	32	708	42	.74
			3	1034	30	1039	34	1035	31	.38
02	20	110	1	724	47	656	49	699	45	
			2	1881	60	1884	62	1931	61	
			3	5199	46	4941	47	5253	46	
03	20	115	1	985	47	992	47	975	50	
			2	9223	58	9524	49	9423	48	
			3	38195	38	38248	38	38304	38	
04	200	125	1	287	44	290	43	288	35	
			2	2371	40	2609	36	2672	46	
			3	12096	36	11587	36	11879	37	
			1							
			2							
			3							
			1							
			2							
			3							

TEST CODE: OT 051589

TEST WATER: OTS OTN20600

CHEM NO. T.E.A.C. Tetra ethylammonium chloride

DATE SAMPLED: —

DATE ELUTED: —

	high conc.	low conc.
INITIAL pH:	7.1	7.2
FINAL pH:	9.8	9.7

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 3

INOCULUM:

Selenastrum ALGA

10^4 CELLS/ML

.1698 MG/L DRY WT.

47.2 MCV

.573 MLS INOCULUM

TEST PREPARATION:

TEST WATERS: MB AAM BATCH T'EN 15800

DILUTION MEDIA: MB

INOCULUM: MB

INOCULATED: MB

SAMPLED: MB / MB

COUNTED: MB/MB

TEST/CONTAINER VOLUME: 50

REPLICATE FLASKS: 1 rep / 6 flasks

DILUTION RANGE: 1000 - .01 mg/L

NOTES: O.T.S. Range finding test

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE OTO51589 CHEM. NUMBER MCN20600

SHAKER NUMBER 204 TEST MATERIAL TEAC tetraethylammonium chloride

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	051589/HB	3:00pm	100	380-420
DAY 1	051689/HB	3:10pm	100	380-420
DAY 2	051789/HB	3:20pm	100	370-410
day 3	051889/HB	2:30pm	100	370-410
DAY 4	051989/mB	3:45pm	100	380-410
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

Controls for: O.T.S

TEST CODE: # OT051584

TREATMENT: T.E.A.C

SPECIES: Selenastrum

CHEM. #: OTN20600

SPIKE: AAM NEG. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	94	1	9779	70	9999	73	9218	72
			2	1373	65	1301	70	1308	63
			3	1599	63	1647	64	1617	62
02	1 20	128	1	6203	58	6179	57	6234	58
			2	5808	56	5951	57	6210	57
			3	9694	51	9933	55	10194	63
03	1 20	113	1	27419	62	25516	60	27969	61
			2	29477	60	31202	59	30298	62
			3	35244	59	37356	57	38714	58
04	1200	67	1	10003	55	10076	55	10594	55
			2	7924	58	8809	59	9380	58
			3	11249	51	12052	53	10539	51
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: Zn POS. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	94	1	552	60	532	59	549	59
			2	839	82	813	81	960	79
			3	1722	63	1347	60	1479	57
02	1 20	128	1	2048	64	1978	62	2133	66
			2	3159	51	3159	50	3064	55
			3	5970	60	6069	61	6298	66
03	1 20	113	1	3294	68	3649	66	4039	66
			2	3837	74	3871	72	[illegible]	[illegible]
			3	25317	60	21509	61	26528	61
04	1200	67	1	1096	58	1144	59	1262	61
			2	1143	48	1145	50	1117	47
			3	12219	53	13049	52	13653	54
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: OTS TEST CODE: OT051589

TREATMENT: TEAC SPECIES: Selenastrum

CHEM. #: OTN20600 SPIKE: 1000 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	194	1	859	68	914	72	879	72
			2						
			3						
02	120	1128	1	3569	60	3748	59	3893	59
			2						
			3						
03	120	1113	1	17585	63	18333	62	18273	63
			2						
			3						
04	200	167	1	6672	58	5970	60	6372	59
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 100 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	193	1	1107	71	1279	72	1208	69
			2						
			3						
02	120	1128	1	4343	57	4478	57	4409	56
			2						
			3						
03	120	1113	1	33359	57	33678	56	33947	56
			2						
			3						
04	200	167	1	7020	57	7794	59	8346	57
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: O.T.S.　　TEST CODE: OT051589

TREATMENT: T.EAC　　SPECIES: Selenastrum

CHEM. #: OTN20600　　SPIKE: 10 mg/L

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	194	1	1769	59	1701	59	1718	59
			2						
			3						
02	1 20	128	1	8494	53	8591	54	9109	55
			2						
			3						
03	1 20	113	1	43888	57	45742	57	44955	57
			2						
			3						
04	200	167	1	14063	46	14716	47	15739	47
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 1 mg/L

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	194	1	11648	53	11683	57	1592	56
			2						
			3						
02	1 20	128	1	16750	53	16717	52	17077	52
			2						
			3						
03	1 20	113	1	41272	55	41939	54	42189	54
			2						
			3						
04	200	167	1	15628	44	16442	44	16593	44
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: O.T.S.

TEST CODE: OT051589

TREATMENT: T.E.A.C.

SPECIES: Selenastrum

CHEM. #: OTN20600

SPIKE: 0.1 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	94	1	17514	56	17658	52	15338	58
			2						
			3						
02	120	128	1	7707	53	7823	53	8038	52
			2						
			3						
03	120	113	1	38413	57	39262	57	40114	57
			2						
			3						
04	1200	67	1	12416	47	12995	47	15269	47
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 0.01 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	94	1	11704	54	11829	57	11824	56
			2						
			3						
02	120	128	1	8344	54	8530	54	8763	54
			2						
			3						
03	120	113	1	41052	57	38591	57	41121	57
			2						
			3						
04	1200	67	1	12249	50	12981	49	13739	49
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

Definative

TEST CODE: SC 060589

TEST WATER: OTS - Phthallic Anhydride

CHEM NO. OTN 23600

DATE SAMPLED:

DATE ELUTED:

	high conc.	low conc.
INITIAL pH:	5.5	8.5
FINAL pH:	5.7	7.5

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 03

INOCULUM:

Selenastrum c	ALGA
~10^4	CELLS/ML
.12	MG/L DRY WT.
32.8	MCV
.39	MLS INOCULUM

TEST PREPARATION:

TEST WATERS: HB AAM BATCH 22800

DILUTION MEDIA: HB

INOCULUM: HB

INOCULATED: HB

SAMPLED: HB

COUNTED: HB

TEST/CONTAINER VOLUME: 50 mls

# REPLICATE FLASKS: 7 concentrations @ 3 reps. ea.

DILUTION RANGE: 20 mg/L - .3 mg/L

NOTES: OTS - definative - not pH adjusted
✓ metal on 22800

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

1000 mg/L

TEST CODE SL060589 CHEM. NUMBER OTN 23600

SHAKER NUMBER 208 TEST MATERIAL Phthallic anhydride

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	060589/HB	2:30	100	370-400
DAY 1	06 06 89/HB	2:00	100	370-400
DAY 2	060789/HB	2:30	96	370-400
day 3	060889/HB	2:45	96	370-390
DAY 4	060989/HB	1:55	96	370-390
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

Controls for:

TEST CODE: P.A.

TREATMENT: OTS

SPECIES: Selenastrum

CHEM. #: OTN23600

SPIKE: AAM NEG. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	188	1	1549	39	1662	45	1654	42
			2	1454	42	1995	42	1579	43
			3	1511	45	1521	41	1552	43
02	120	389	1	6793	46	7184	46	6788	46
			2	7746	45	7411	43	8302	43
			3	7732	41	8259	42	7854	41
03	200	158	1	5716	43	5789	42	5694	44
			2	4372	38	4476	38	4471	38
			3	3974	36	3943	36	3773	36
04	200	134	1	12868	36	12804	37	15878	37
			2	11126	32	11041	31	11186	35
			3	8719	33	8521	34	8470	33
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: Zn POS. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	188	1	417	57	452	53	435	47
			2	873	41	852	43	856	49
			3	1657	48	1034	48	1081	44
02	120	389	1	1886	60	1822	61	976	57
			2	3538	68	3703	64	3526	67
			3	7490	42	7096	51	7569	52
03	200	158	1	208	42	226	63	267	[illegible]
			2	2778	49	2244	44	2384	50
			3	4850	50	5102	50	5009	55
04	200	134	1	298	55	304	57	366	61
			2	4636	50	4707	48	4698	48
			3	13110	42	12988	43	12338	45
			1						
			2						
			3						
			1						
			2						
			3						

1.48
.74
.38

TEST WATER: OTS TEST CODE: P.A.

TREATMENT: pH 5.5 SPECIES: Selenastrum

CHEM. #: OTN23600 SPIKE: 20 mg/L

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	188	1	278	59	289	54	262	57
			2	363	79	296	72	312	64
			3	303	48	350	60	367	47
02	120	3819	1	1784	68	1753	73	1656	65
			2	1173	64	997	70	1116	62
			3	635	56	658	59	596	60
03	200	1518	1	275	61	309	51	314	61
			2	229	47	183	53	199	42
			3	732	49	646	49	674	50
04	200	1314	1	330	57	378	54	402	48
			2	416	48	473	50	388	49
			3	811	43	774	48	719	44
			1						
			2						
			3						
			1						
			2						
			3						

INITIAL
pH 6.8

SPIKE: 10 mg/L

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	188	1	1402	38	1418	38	1349	38
			2	1583	37	1627	37	1629	38
			3	1371	38	1432	37	1471	40
02	120	3819	1	6748	37	6393	36	7296	36
			2	7505	37	8055	37	7866	37
			3	7734	37	7497	36	7658	36
03	200	1518	1	3139	31	3432	31	3242	32
			2	2914	30	2862	30	3117	31
			3	2644	31	2853	30	2998	30
04	200	1314	1	5657	34	6025	33	5956	33
			2	4979	33	4809	33	5062	32
			3	5353	33	5287	32	5438	33
			1						
			2						
			3						
			1						
			2						
			3						

FINAL
pH 7.4

TEST WATER: OTS TEST CODE: P.A.

TREATMENT: SPECIES: Selenastrum

CHEM. #: OTN23600 SPIKE: 5 mg/L.

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	188	1	11203	39	11250	38	11295	39
			2	11284	38	11234	37	11338	38
			3	11674	40	11478	38	11785	37
02	1 20	389	1	16834	35	17089	36	17309	35
			2	18125	39	18316	38	18437	38
			3	18456	39	18828	37	18666	38
03	1200	1158	1	12481	30	12679	32	12687	31
			2	13557	30	13792	32	13738	31
			3	13611	29	13563	31	13491	3[illegible]
04	1200	1134	1	14548	33	14417	32	14457	32
			2	17395	31	17431	32	17543	32
			3	15948	33	16019	32	16124	33
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 2.5 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	188	1	11546	41	11529	37	11582	39
			2	11159	41	11234	39	11339	40
			3	715	42	1640	37	1693	42
02	1 20	389	1	18485	37	18768	39	18788	39
			2	18055	36	18237	37	18012	37
			3	14197	42	14080	40	14391	39
03	1200	1158	1	13719	33	13766	32	13908	33
			2	13141	31	13289	30	13244	31
			3	11874	38	12261	36	12619	37
04	1200	1134	1	18612	31	18886	30	19042	31
			2	16879	33	16084	33	16079	33
			3	15739	34	16328	35	16648	35
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: OTS TEST CODE: P.A.

TREATMENT: SPECIES: Selenastrum

CHEM. #: OTN23600 SPIKE: 1.25 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	818	1	1288	37	1316	38	1310	40
			2	1171	38	1226	36	1228	36
			3	975	35	1049	39	1028	36
02	1210	389	1	6948	36	7555	36	7576	36
			2	7278	36	7044	35	7517	35
			3	4648	35	4948	36	5279	34
03	2000	1518	1	2203	32	2696	30	2827	30
			2	3233	31	3155	29	3230	30
			3	1557	33	1461	36	1489	34
04	2010	134	1	6355	33	6384	31	6495	30
			2	5837	32	5647	33	5539	30
			3	4389	34	4352	31	4017	32
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: .625 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	818	1	1422	39	1392	39	1381	36
			2	1536	39	1516	38	1577	37
			3	1165	39	1190	38	1221	38
02	1210	389	1	8137	39	8048	38	8271	38
			2	8406	36	8430	35	8588	36
			3	7239	36	7443	36	7414	36
03	2000	1518	1	3901	33	3738	33	3876	32
			2	3052	29	3186	29	3226	29
			3	3263	33	3437	33	3403	32
04	2010	134	1	8302	32	8218	33	8410	31
			2	9845	33	8941	34	9171	33
			3	9239	33	9842	33	8151	33
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: OTS

TEST CODE: P.A.

TREATMENT:

SPECIES: Selenastrum

CHEM. #: OTN23600

SPIKE: .312 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	188	1	11290	41	11247	43	11257	39
			2	11774	39	11893	39	11899	38
			3	11855	36	11396	38	11504	35
02	1210	3819	1	7311	36	7283	36	7651	36
			2	7809	37	8456	38	8506	37
			3	8274	38	7993	37	8113	36
03	2010	1518	1	3034	32	3047	30	3181	32
			2	3273	31	3285	30	3447	31
			3	3625	32	3456	32	3588	32
04	2100	1314	1	10291	33	10937	31	11129	32
			2	8328	32	8961	33	8867	33
			3	9211	31	7811	33	7724	31
			1						
			2						
			3						
			1						
			2						
			3						

~~SPIKE:~~

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

range Finding

TEST CODE: SC052389A

TEST WATER: OTS - Phthallic Anhydride (P.A.)

CHEM NO. OTA21600

DATE SAMPLED: —

DATE ELUTED: —

	high conc.	low conc.
INITIAL pH:	5.4	7.2
FINAL pH:	7.17	9.79

STOCK CULTURE DATA:

MEDIA: AMM

DAYS GROWTH: 04

INOCULUM:

Selenastrum c.	ALGA
~10^4	CELLS/ML
.18	MG/L DRY WT.
50	MCV
.52	MLS INOCULUM

TEST PREPARATION:

TEST WATERS: NB AAM BATCH MEN18800

DILUTION MEDIA: NB

INOCULUM: NB

INOCULATED: NB

SAMPLED: NB

COUNTED: NB

TEST/CONTAINER VOLUME: 50/125

REPLICATE FLASKS: 1

DILUTION RANGE: 1000 - .01 mg/L

NOTES: Special O.T.S. Procedure. Range Finding Test.

* First 2 concentrations were pH adjusted; remainder were not. Ph adjustment appeared to decrease vicity in the R.F.T.

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE SC052389A CHEM. NUMBER OTN21600

SHAKER NUMBER 211 TEST MATERIAL Phthallic Anhydride

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	052389/ HB	1:00 pm	100	370-420
DAY 1	052489/HB	1:20pm	100	370-410
DAY 2	052589/HB	1:10pm	100	370-410
day 3	052689/MB	2:30pm	100	360-440
DAY 4	052789/HB	11:45pm	100	360-420
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

SE0573891

[illegible]: Controls for: Phthalic Anhydride TEST CODE: SE

TREATMENT: N/A SPECIES: Selenastrum

CHEM. #: OTA 21600 SPIKE: AAM NEG. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	140	1	1126	65	1139	68	1199	63
			2	1451	52	1522	52	1521	52
			3	1935	53	2024	54	1926	49
02	1 20	52	1	3270	70	3348	71	3221	71
			2	6248	57	6679	55	6874	55
			3	5758	55	6281	54	6552	54
03	200	1118	1	1953	62	1978	65	1012	60
			2	4969	59	5064	61	5033	60
			3	4787	60	5031	60	4988	60
04	200	178	1	2490	57	2645	58	2595	59
			2	14743	44	14965	45	15049	43
			3	12519	50	12665	50	12822	49
			1						
			2						
			3						
			1						
			2						
			3						

CHEM #

SPIKE: Zn POS. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV	
01	1 20	140	1	573	65	625	61	642	56	1.48
			2	592	76	614	72	622	67	.74
			3	976	58	1022	65	1034	57	.37
02	1 20	52	1	531	73	597	68	549	71	
			2	1024	61	1044	58	1023	61	
			3	3094	54	3073	49	3256	45	
03	200	1118	1	147	67	174	71	194	80	
			2	1410	50	1400	47	1421	45	
			3	4662	50	4683	51	4962	49	
04	200	178	1	300	61	538	50	306	66	
			2	2108	55	2210	56	2317	58	
			3	9721	53	10317	52	10102	52	
			1							
			2							
			3							
			1							
			2							
			3							

TEST WATER: PHTHALIC ANHYDRIDE　　TEST CODE: SC052389A

TREATMENT: N/A　　SPECIES: Selenastrum

CHEM. #: OTA21600　　SPIKE: 1000 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	40	1	522	82	497	79	570	63
			2						
			3						
02	20	52	1	526	60	421	59	409	61
			2						
			3						
03	20	118	1	2558	36	2839	41	2785	40
			2						
			3						
04	20	78	1	8958	27	9879	26	9619	28
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 100 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	40	1	876	55	1006	53	1051	50
			2						
			3						
02	20	52	1	2275	49	2294	49	2368	48
			2						
			3						
03	200	118	1	4466	47	4457	47	4391	49
			2						
			3						
04	200	78	1	13314	40	13164	39	14181	40
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: PHTHALIC ANHYDRIDE

TEST CODE: SC052389A

TREATMENT: N/A

SPECIES: Selenastrum

CHEM. #: OTN21600

SPIKE: 10 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	40	1	813	75	909	76	910	73
			2						
			3						
02	20	52	1	1929	66	1995	68	2018	65
			2						
			3						
03	200	118	1	932	60	927	61	972	60
			2						
			3						
04	200	78	1	1917	68	2015	65	2012	66
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

?

SPIKE: 1 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	40	1	908	60	822	63	965	63
			2						
			3						
02	20	52	1	2655	59	2719	69	2703	66
			2						
			3						
03	200	118	1	783	57	870	65	767	59
			2						
			3						
04	200	78	1	1457	61	1517	62	1496	61
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

?

TEST WATER: Phthalic Anhydride TEST CODE: SC052389A

TREATMENT: N/A SPECIES: Selenastrum

CHEM. #: DTN21600 SPIKE: 0.1 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	40	1	702	54	699	49	825	53
			2						
			3						
02	20	52	1	2217	47	2301	48	2343	45
			2						
			3						
03	200	118	1	2343	45	2335	46	2462	44
			2						
			3						
04	200	78	1	6593	45	7192	46	7029	46
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 0.01 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	40	1	1166	56	1152	45	1177	52
			2						
			3						
02	20	52	1	4856	53	5029	54	5173	54
			2						
			3						
03	200	118	1	4421	64	4407	64	4436	63
			2						
			3						
04	200	78	1	17356	53	17032	53	17845	53
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST CODE: SC062689A

TEST WATER: Azosulfamide

CHEM NO. OTN 2560D

DATE SAMPLED: N/A

DATE ELUTED: N/A

	high conc.	low conc.
INITIAL pH:	7.2	7.3
FINAL pH:	7.2	8.4

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 4

INOCULUM:

Selenastrum	ALGA
10^4	CELLS/ML
0.1303	MG/L DRY WT.
36.2	MCV
0.3447	MLS INOCULUM

TEST PREPARATION:

TEST WATERS: SS AAM BATCH MEN268OD

DILUTION MEDIA: SS

INOCULUM: SS

INOCULATED: SS

SAMPLED:

COUNTED:

TEST/CONTAINER VOLUME: 50/125

# REPLICATE FLASKS: 3

DILUTION RANGE: 100 - 3.12 mg/L

NOTES: Definitive test; procedure as per Bollman memo 013084 except #5

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE SC062689 A CHEM. NUMBER OTW 25600

SHAKER NUMBER 209 TEST MATERIAL Azosulfamide

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	6-26-89 AS	1820h	110	420-440
DAY 1	6-27-89 AS	1550h	110	360-390
DAY 2	062889/HB	5:15pm	100	370-390
day 3	062989/HB	5:00pm	100	360-410
DAY 4	063089/HB	4:45pm	100	360-410
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

Controls for:

TEST CODE: ~~DC~~ SC062289A-C

TREATMENT:

SPECIES: Selenastrum

CHEM. #: MEW 268D

SPIKE: AAM NEG. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	242	1	590	55	594	62	631	59
			2	604	59	643	59	636	62
			3	630	59	728	62	1155	62
02	20	184	1	979	65	838	69	940	66
			2	1479	72	1438	67	1539	65
			3	1709	66	1738	69	1783	68
03	20	1319	1	7378	71	7148	71	7548	69
			2	7039	75	8050	76	8144	78
			3	8631	75	8740	75	9144	76
04	20	2109	1	33491	55	35211	58	35264	58
			2	47812	56	48525	56	49691	58
			3	44168	58	36194	58	42[illegible]	58
			1						
			2						
			3						
			1						
			2						
			3						

6312605

SPIKE: Zn POS. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 2 10	293	1	645	53	841	58	822	57
			2	612	50	669	49	693	49
			3	842	62	907	49	1086	72
02	20	184	1	972	61	939	57	1099	56
			2	1129	45	1113	50	1162	49
			3	990	61	514	72	573	66
03	20	134	1	5739	65	5431	68	5819	61
			2	4918	61	4607	59	4757	59
			3	1905	55	1713	54	1774	58
04	20	209	1	44029	49	44140	49	44589	49
			2	12321	71	13098	70	12538	69
			3	3040	68	2092	70	2122	72
			1						
			2						
			3						
			1						
			2						
			3						

.5 ml
1 ml
2 ml

TEST WATER: Azo sulfamide

TEST CODE: SC062689A

TREATMENT:

SPECIES: Selenastrum

CHEM. #: OTN256D

SPIKE: 10 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
011	120	273	1	741	64	751	48	898	53
			2	1194	59	817	50	789	51
			3	927	50	933	49	1079	50
012	120	184	1	1397	47	1582	46	1671	46
			2	1409	48	1678	46	1441	51
			3	1099	47	1164	47	1054	48
013	120	134	1	4245	54	4124	53	4294	54
			2	5116	53	5039	52	5088	52
			3	5436	51	5211	50	5849	50
014	120	209	1	10005	70	10901	74	10178	70
			2	13884	62	13962	60	13955	60
			3	15257	55	15626	55	15603	55
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 50 mg/l

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
011	120	293	1	725	51	741	56	693	56
			2	1136	59	709	50	709	49
			3	911	51	849	55	832	64
012	120	184	1	1089	53	1107	52	1021	52
			2	1049	51	1027	51	1072	51
			3	1111	50	1201	50	1206	47
013	120	134	1	5348	59	5107	57	5649	59
			2	5630	58	5389	57	5721	57
			3	5668	52	5701	51	5939	53
014	120	209	1	18336	64	18028	64	18647	64
			2	21467	73	22362	72	22222	73
			3	21056	69	21987	67	20759	69
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: Azo sulfamide

TEST CODE: SC 062689 A

TREATMENT:

SPECIES: Selenastrum

CHEM. #: OTN 256D

SPIKE: 25 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
011	1 200	295	1	7211	62	8216	53	7911	59
			2	6570	52	7705	48	7225	48
			3	7993	53	7769	52	7992	54
012	1 200	184	1	11029	55	11619	54	11092	55
			2	11105	55	11013	57	11033	57
			3	9180	62	9888	58	9580	57
013	1 200	134	1	51410	60	52161	59	51171	60
			2	26216	50	29111	51	27743	51
			3	48014	71	47728	72	45161	72
014	1 200	209	1	20223	57	20557	56	20671	57
			2	15477	46	16801	47	16049	46
			3	23509	62	24016	61	24301	62
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 12.5 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
011	1 200	293	1	7272	57	7211	52	7770	62
			2	9112	54	9107	54	11104	51
			3	6770	55	9072	64	8511	62
012	1 200	184	1	11105	55	11013	57	11111	57
			2	7917	60	8377	62	9320	61
			3	11448	71	11424	68	11789	68
013	1 200	134	1	44211	73	47211	72	42516	75
			2	8456	79	8116	77	8322	76
			3	8677	74	8412	74	8769	76
014	1 200	209	1	27992	61	26798	62	27028	62
			2	24467	65	25118	64	24361	64
			3	28333	69	27637	64	28648	62
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: Azo sulfamide

TEST CODE: SC 062689 A

TREATMENT:

SPECIES: [illegible]

CHEM. #: OTN 256 D

SPIKE: 6.25 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
011	1 20	283	1	795	55	820	60	833	58
			2	613	53	735	52	718	54
			3	607	55	712	59	759	56
012	1 20	1184	1	909	64	852	60	996	61
			2	1007	61	1009	62	1100	60
			3	635	60	609	59	654	56
013	1 20	1134	1	5111	74	5397	74	4646	69
			2	6108	75	6377	75	6069	74
			3	6681	73	6794	74	7170	75
014	1 20	2019	1	25965	57	26475	60	27731	60
			2	31547	59	31759	58	32530	58
			3	35507	57	36174	56	35102	58
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 3.12 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
011	1 20	293	1	629	61	569	58	659	68
			2	595	52	778	54	764	56
			3	606	54	439	56	656	61
012	1 20	1184	1	814	70	806	66	894	66
			2	544	60	548	52	561	54
			3	778	65	760	68	744	67
013	1 20	1134	1	8547	73	8362	74	8552	71
			2	6591	73	6314	74	6701	73
			3	13594	71	12116	74	13874	71
014	1 20	2019	1	41772	61	46970	61	42716	61
			2	36318	61	36844	61	36753	61
			3	36372	65	32011	65	33250	64
			1						
			2						
			3						
			1						
			2						
			3						

TEST CODE: SC 061989 A-C

TEST WATER: OTS - [redacted]lycidol, N-octylamine

CHEM NO. OTN25600-602

DATE SAMPLED: ______

DATE ELUTED: ______

	high conc.	low conc.
INITIAL pH:		
FINAL pH:		

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 04

INOCULUM:

Selenastrum c. ALGA

~10^4 CELLS/ML

0.1454 MG/L DRY WT.

40.4 MCV

.36 MLS INOCULUM

TEST PREPARATION:

TEST WATERS: WB AAM BATCH 23800

DILUTION MEDIA: WB

INOCULUM: WB

INOCULATED: WB

SAMPLED: WB

COUNTED: WB

TEST/CONTAINER VOLUME: ______

# REPLICATE FLASKS: ______

DILUTION RANGE: ______

NOTES: OTS Range Finding Tests

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE SC061989A-C CHEM. NUMBER OTN25600-602

SHAKER NUMBER ________ TEST MATERIAL ________

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	061989/SHB	2:30 pm	96	390-420
DAY 1	062089/SHB	2:00 pm	96	390-420
DAY 2	062189/SHB	2:45 pm	96	390-420
day 3	062289/SHB	2:00 pm	96	390-410
DAY 4	062389/SHB	2:15 pm	96	390-410
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

TEST WATER: Controls for:　　TEST CODE: DC

TREATMENT:　　SPECIES: Selenastrum

CHEM. #: OTN25600-602　　SPIKE: AAM NEG. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	92	1	643	75	618	82	664	79
			2	711	77	709	78	699	80
			3	798	82	771	82	839	87
02	1 20	1/59	1	1800	81	2253	78	2004	80
			2	2811	70	2663	73	2968	70
			3	3628	71	3811	71	3915	71
03	200	21/12	1	1167	65	1111	71	1081	71
			2	1362	55	1196	72	1241	72
			3	1341	67	1414	65	1345	69
04	1200	1/16	1	4122	30	4221	30	4504	29
			2	5720	31	5761	30	5747	30
			3	5222	33	5733	32	6011	37
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: Zn POS. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	92	1	493	51	479	46	452	43
			2	552	67	603	74	619	69
			3	773	83	703	88	709	90
02	1 23	1/59	1	528	44	498	42	4029	43
			2	1709	65	1306	67	1709	45
			3	2032	73	2054	74	1985	73
03	1 20	2/12	1	772	51	866	49	731	55
			2	7931	60	7811	60	8151	59
			3	7883	88	8502	47	7895	83
04	1 223	1/26	1	1184	45	921	33	157	34
			2	3214	29	3137	29	3144	28
			3	3971	29	4155	28	4563	29
			1						
			2						
			3						
			1						
			2						
			3						

1.45
.74
.38

TEST WATER: ______________________ TEST CODE: ______________________

TREATMENT: ______________________ SPECIES: Selenastrum

OTN 25600

CHEM. #: Azosulfamide SPIKE: 1000 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	192	1	1749	55	1827	59	1798	55
			2						
			3						
02	1 20	1159	1	22375	37	24445	34	22615	35
			2						
			3						
03	1 20	2112	1	27378	54	27887	52	27550	54
			2						
			3						
04	1 20	1106	1	53774	23	52618	23	53610	23
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 100 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	192	1	769	61	777	57	572	61
			2						
			3						
02	1 20	1159	1	22570	45	17555	45	21195	44
			2						
			3						
03	1 20	2112	1	51142	59	54610	60	53113	57
			2						
			3						
04	1 20	1106	1	91019	32	98112	32	9751	32
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: ______________________ TEST CODE: ______________

TREATMENT: ______________________ SPECIES: Selenastrum

CHEM. #: Azosulfamide SPIKE: 10 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	192	1	702	68	694	71	698	69
			2						
			3						
02	1 20	159	1	1448	64	1691	66	1392	70
			2						
			3						
03	1 200	2112	1	5377	74	5270	74	5367	73
			2						
			3						
04	200	1016	1	2818	31	3047	31	3374	31
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 1 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	192	1	540	76	617	73	669	76
			2						
			3						
02	1 20	159	1	2635	74	2754	70	2719	71
			2						
			3						
03	1 200	2112	1	1438	65	1277	71	1356	68
			2						
			3						
04	200	1016	1	4188	28	4259	29	4367	30
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: ____________________ TEST CODE: ____________________

TREATMENT: ____________________ SPECIES: Selenastrum

CHEM. #: Azosulfamide SPIKE: .1 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	192	1	5729	76	6652	70	6460	70
			2						
			3						
02	1 210	1159	1	24555	65	23371	64	25310	65
			2						
			3						
03	200	2112	1	13222	69	12612	65	19218	66
			2						
			3						
04	200	1016	1	15358	27	15944	28	15929	27
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 0.01 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 210	192	1	5814	72	6119	64	6528	73
			2						
			3						
02	1 210	1159	1	11920	55	22512	54	11848	61
			2						
			3						
03	200	2112	1	10182	70	12114	69	12115	66
			2						
			3						
04	2019	1066	1	41192	34	42238	36	14230	37
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST CODE: SC062689B

TEST WATER: glycidol

CHEM NO. OTN 25601

DATE SAMPLED: N/A

DATE ELUTED: N/A

	high conc.	low conc.
INITIAL pH:	78	7,3
FINAL pH:	7,3	8,0

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH:

INOCULUM:

Selenastrum	ALGA
10^4	CELLS/ML
,1303	MG/L DRY WT.
36.2	MCV
0.3447	MLS INOCULUM

TEST PREPARATION:

TEST WATERS: SS AAM BATCH MEN 26800

DILUTION MEDIA: SS

INOCULUM: SS

INOCULATED: SS

SAMPLED:

COUNTED:

TEST/CONTAINER VOLUME: 50/125

# REPLICATE FLASKS: 3

DILUTION RANGE: 500 - 31.25 mg/L

NOTES: Definitive test; procedure as per Bollman memo 013089

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE SC062689 A CHEM. NUMBER OTW 2560D

SHAKER NUMBER 209 TEST MATERIAL Azosulfamide / GLYCIDOL / N-OCTYLAMINE

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	6-26-89 [illegible]	1820L	110	420-440
DAY 1	6-27-89 [illegible]	1550L	110	360-390
DAY 2	062889/JHB	5:15pm	100	370-390
day 3	062989/JHB	5:00pm	100	360-410
DAY 4	063089/JHB	4:45pm	100	360-410
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

TEST WATER: Glycidol

TEST CODE: SC062689 B

TREATMENT:

SPECIES: [illegible]

CHEM. #: ws OTN25601

SPIKE: 500 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
011	20	293	1	853	49	1041	63	1208	62
			2	930	58	976	51	1090	56
			3	1249	49	923	53	1020	55
012	20	184	1	988	60	926	57	987	58
			2	848	61	911	60	912	61
			3	630	43	621	44	681	44
013	20	134	1	748	49	741	49	811	45
			2	582	42	613	41	531	44
			3	543	45	562	44	526	41
014	20	209	1	1553	42	1549	41	1560	43
			2	1093	48	1114	47	1231	45
			3	992	49	978	50	963	48
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 250 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
011	20	293	1	790	54	901	54	990	49
			2	567	43	559	47	610	46
			3	1022	53	1189	56	1144	46
012	20	184	1	1162	61	1114	60	632	57
			2	1669	62	1764	62	1559	60
			3	1260	60	1169	57	1172	60
013	20	134	1	2384	84	2761	82	2338	81
			2	1792	83	1699	82	1714	85
			3	3550	89	3478	86	3451	90
014	20	209	1	9307	61	9249	61	9520	61
			2	8326	68	8411	67	8338	67
			3	14876	66	15126	65	15639	67
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: Glycidal

TEST CODE: SC062689 B

TREATMENT:

SPECIES: Selenastrum

CHEM. #: OTN25601

SPIKE: 125 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	293	1	760	53	760	55	757	56
			2	749	64	762	69	940	60
			3	514	45	494	48	554	51
02	1 20	184	1	1147	59	1108	59	1178	58
			2	1118	55	1201	56	1095	59
			3	1228	58	1201	58	1251	55
03	1 20	134	1	9136	58	8999	57	9136	57
			2	9389	60	9173	59	9151	57
			3	9389	53	9971	52	9566	52
04	1 20	209	1	33625	64	33559	64	38203	64
			2	41716	52	41171	51	40582	52
			3	31919	52	32047	51	321 58	52
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 62.5 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	293	1	906	57	973	51	978	60
			2	610	46	713	54	721	56
			3	932	55	885	65	894	56
02	1 20	184	1	1233	66	1281	64	1280	65
			2	1104	68	1173	68	1144	66
			3	1345	72	1303	68	1299	69
03	1 20	134	1	7392	72	7716	72	7146	71
			2	6695	73	6917	72	6460	72
			3	6522	72	6793	71	6338	73
04	1 20	209	1	46818	57	47083	56	48782	57
			2	48752	58	46716	57	49609	57
			3	53342	59	52611	58	53486	58
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: Glycidal

TEST CODE: SC062689 B

TREATMENT:

SPECIES: Selenastrum

CHEM. #: OTN25601

SPIKE: 31.25 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	793	1	919	57	852	59	801	55
			2	710	51	787	56	866	63
			3	671	61	715	56	702	64
02	120	1189	1	11249	62	12513	61	11813	66
			2	8882	77	9388	75	8773	77
			3	11231	73	12026	72	11449	77
03	120	1314	1	7445	67	7609	67	7161	67
			2	14184	76	13886	76	14216	75
			3	15670	76	14911	76	17039	77
04	120	2019	1	35373	56	36760	57	37105	56
			2	39290	58	40277	58	40418	59
			3	51282	59	50981	59	52151	62
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE:

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01			1						
			2						
			3						
02			1						
			2						
			3						
03			1						
			2						
			3						
04			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST CODE: SC 061989 A-C

TEST WATER: OTS - azosulfamide, [redacted]-octylamin.

CHEM NO. OTN25600-602

DATE SAMPLED: ____

DATE ELUTED: ____

	high conc.	low conc.
INITIAL pH:		
FINAL pH:		

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 04

INOCULUM:

Selenastrum c.	ALGA
$\sim 10^4$	CELLS/ML
0.1454	MG/L DRY WT.
40.4	MCV
.36	MLS INOCULUM

TEST PREPARATION:

TEST WATERS: WB AAM BATCH 23800

DILUTION MEDIA: WB

INOCULUM: WB

INOCULATED: WB

SAMPLED: HB

COUNTED: HB

TEST/CONTAINER VOLUME: ____

# REPLICATE FLASKS: ____

DILUTION RANGE: ____

NOTES: OTS Range Finding Tests

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE SC061989A-C CHEM. NUMBER OTN25600-602

SHAKER NUMBER ______ TEST MATERIAL ______

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	061989/JHB	2:30pm	96	390-420
DAY 1	062089/JHB	2:00pm	96	390-420
DAY 2	062189/JHB	2:45 pm	96	390-420
day 3	062289/JHB	2:00 pm	96	390-410
DAY 4	062389/JHB	2:15pm	96	390-410
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

TEST CODE: DC

TREATMENT:

SPECIES: Selenastrum

CHEM. #: OTN25600-602

SPIKE: AAM NEG. CONTROL

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	92	1	643	75	618	82	664	79
			2	711	77	709	78	699	80
			3	798	82	771	85	839	81
02	120	159	1	1800	81	2253	78	2004	80
			2	3811	70	2663	73	2468	72
			3	3628	71	3811	71	3915	71
03	200	2112	1	1167	65	1111	71	1081	71
			2	1362	52	1196	72	1241	72
			3	1341	67	1414	65	1345	69
04	1200	1016	1	4122	30	4231	30	4504	29
			2	5727	31	5761	30	5747	30
			3	5322	33	5763	32	6011	31
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: Zn POS. CONTROL

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV	
01	120	92	1	493	51	479	46	452	43	1.48
			2	592	67	603	74	619	69	.74
			3	723	83	703	88	709	90	.38
02	120	159	1	528	44	498	42	479	43	
			2	1709	65	1306	67	1709	65	
			3	2032	73	2054	74	1985	73	
03	1202	112	1	772	51	866	49	731	55	
			2	7931	60	7811	60	8151	59	
			3	7883	88	8502	82	7895	83	
04	222	1216	1	1784	45	921	33	157	34	
			2	3214	27	3137	29	3144	21	
			3	3971	59	4155	27	4563	29	
			1							
			2							
			3							
			1							
			2							
			3							

TEST WATER: ______________ ______

TEST CODE: ______________

TREATMENT: ______________

SPECIES: Selenastrum

OTN 25601

CHEM. #: Glycidol

SPIKE: 1000 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	192	1	587	47	530	40	613	43
			2						
			3						
02	20	1159	1	802	38	857	37	852	38
			2						
			3						
03	20	2112	1	1253	41	1340	43	1540	43
			2						
			3						
04	20	1326	1	471	212	3413	210	3175	210
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 100 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	192	1	672	75	678	75	658	77
			2						
			3						
02	20	1159	1	1923	71	1918	71	1889	74
			2						
			3						
03	26	2112	1	7677	79	7689	80	7727	78
			2						
			3						
04	200	1106	1	3501	30	3578	30	3726	27
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: ______

TEST CODE: ______

TREATMENT: ______

SPECIES: Selenastrum

CHEM. #: glycidol

SPIKE: 10 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 210	192	1	1654	77	1683	76	1679	77
			2						
			3						
02	1 120	159	1	11857	72	21146	72	19111	71
			2						
			3						
03	1 200	2112	1	10460	79	9814	80	10385	81
			2						
			3						
04	200	106	1	15156	30	14743	30	15083	30
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 1 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 210	192	1	1687	75	1659	80	1697	80
			2						
			3						
02	1 1210	159	1	20919	70	27510	68	29641	68
			2						
			3						
03	1200	2112	1	13912	69	14115	70	15419	68
			2						
			3						
04	200	106	1	49136	29	53410	29	50410	29
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: ______________________ TEST CODE: ______________________

TREATMENT: ______________________ SPECIES: Selenastrum

CHEM. #: glycidol SPIKE: .1 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	192	1	4617	23	4619	58	5419	28
			2						
			3						
02	1 20	1519	1	11609	59	11741	62	11647	60
			2						
			3						
03	2100	2112	1	1895	63	11114	63	11093	60
			2						
			3						
04	2200	1016	1	4977	28	39311	29	43312	29
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: .01 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	192	1	5319	64	5538	67	5417	65
			2						
			3						
02	1 20	1519	1	11509	59	11543	64	11593	61
			2						
			3						
03	200	2112	1	11041	64	11201	63	11059	59
			2						
			3						
04	200	1016	1	3986	30	39812	33	36571	35
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST CODE: SC062689C

TEST WATER: N-octylamine

CHEM NO. OTN25602

DATE SAMPLED: N/A

DATE ELUTED: N/A

	high conc.	low conc.
INITIAL pH:	7.5	7.3
FINAL pH:	7.5	8.1

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 4

INOCULUM:

Selenastrum ALGA

10^4 CELLS/ML

0.1303 MG/L DRY WT.

36.2 MCV

0.3447 MLS INOCULUM

TEST PREPARATION:

TEST WATERS: SB AAM BATCH MEN2680

DILUTION MEDIA: SB

INOCULUM: SB

INOCULATED: SB

SAMPLED:

COUNTED:

TEST/CONTAINER VOLUME: 50/125

# REPLICATE FLASKS: 3

DILUTION RANGE: 1 - 0.0312 mg/L

NOTES: Definitive test; procedure as per Ballman memo 013089 except #5

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE SC062689 A CHEM. NUMBER OTN 2560D

SHAKER NUMBER 209 TEST MATERIAL Acesulfame/GLYCIDOL/N-OCTYLAMINE

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	6-26-89	1820 L	100	420-440
DAY 1	6-27-89	1550 L	100	360-390
DAY 2	062889/KB	5:15pm	100	370-390
day 3	062989/KB	5:00pm	100	360-410
DAY 4	063089/KB	4:45pm	100	360-410
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

TEST WATER: N-octylamine

TEST CODE: SC062689C

TREATMENT:

SPECIES: Selenastrum

CHEM. #: OTN25602

SPIKE: 1 mg/L

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	293	1	8018	76	11098	65	1193	78
			2	659	58	738	51	746	60
			3	705	63	693	59	624	57
02	1 20	184	1	1091	62	1116	61	1110	60
			2	1045	60	1056	63	998	63
			3	1274	61	1285	61	1711	60
03	1 20	134	1	1458	57	1436	57	1443	57
			2	1097	52	1161	51	1087	56
			3	1935	53	1748	54	2065	54
04	1 20	209	1	2271	51	2313	51	2249	52
			2	1771	56	1698	56	1411	55
			3	1867	52	1906	51	1968	53
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 0.5 mg/L

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	293	1	709	64	745	56	796	64
			2	498	57	492	56	588	59
			3	649	60	622	60	655	58
02	1 20	184	1	1445	65	1479	65	1398	65
			2	656	58	639	60	592	60
			3	598	59	636	54	701	55
03	1 20	134	1	1554	55	1613	54	1423	53
			2	3122	53	3001	52	3280	56
			3	5668	54	5491	53	5796	56
04	1 20	209	1	6101	56	5981	57	6165	57
			2	6550	51	6567	51	6611	50
			3	5133	52	5418	51	5301	54
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: N-octylamine TEST CODE: SC062689C

TREATMENT: SPECIES: Selenastrum

CHEM. #: OTN 25602 SPIKE: 0.25 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	2913	1	1644	58	715	58	767	59
			2	580	53	705	52	763	65
			3	596	55	629	57	629	56
02	120	1184	1	1413	65	1341	70	1404	67
			2	886	61	913	60	908	63
			3	784	61	817	60	727	65
03	120	1314	1	5088	63	5106	60	5111	60
			2	3613	61	3805	60	3464	61
			3	5528	63	5497	63	5422	62
04	120	2019	1	22358	54	23007	53	21974	53
			2	25731	55	26940	53	26354	54
			3	18898	54	19580	54	19953	54
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 0.125 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	2913	1	620	57	634	64	677	64
			2	718	62	693	63	711	64
			3	675	66	729	66	746	64
02	120	1184	1	1174	68	1246	72	1283	69
			2	1395	68	1402	67	1331	67
			3	2006	73	2016	68	1987	67
03	120	1314	1	8352	69	8413	68	8250	68
			2	17553	73	15991	74	17930	73
			3	11385	69	9978	70	11256	76
04	120	2019	1	38934	54	40213	53	39597	55
			2	48638	55	47697	54	49439	55
			3	51773	55	49028	54	53325	56
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: N-octylamine TEST CODE: SC062689C

TREATMENT: SPECIES: Selenastrum

CHEM. #: OTN25602 SPIKE: 0.0625 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	793	1	598	53	699	58	697	59
			2	1119	41	1427	41	1542	41
			3	609	59	761	52	738	55
02	20	184	1	1149	65	1213	64	1181	62
			2	1583	59	1401	60	1435	58
			3	1292	68	1297	65	1268	67
03	20	134	1	4150	49	5007	50	3929	48
			2	8720	70	8666	69	8814	70
			3	5097	62	5178	62	6013	62
04	20	209	1	31653	57	32810	58	31949	60
			2	51823	51	54719	53	22409	51
			3	48495	57	49665	57	50560	57
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 0.03125 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	793	1	986	48	1026	45	983	47
			2	581	64	705	52	909	63
			3	789	58	1022	58	966	60
02	20	184	1	1341	58	1407	59	1366	60
			2	674	56	659	57	761	58
			3	1107	55	1500	59	1158	56
03	20	134	1	11845	72	11960	73	11982	72
			2	7761	69	10211	70	9978	69
			3	7435	74	7459	73	7660	74
04	20	209	1	37730	53	38696	53	39769	54
			2	58231	49	57827	50	58214	51
			3	55067	59	55803	59	57370	59
			1						
			2						
			3						
			1						
			2						
			3						

TEST CODE: SC 061989 A-C

TEST WATER: OTS - azosulfamide, glycidol, [redacted]

CHEM NO. OTN25600-602

DATE SAMPLED: ____

DATE ELUTED: ____

	high conc.	low conc.
INITIAL pH:		
FINAL pH:		

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 04

INOCULUM:

Selenastrum c. ALGA

~10^4 CELLS/ML

0.1454 MG/L DRY WT.

40.4 MCV

.36 MLS INOCULUM

TEST PREPARATION:

TEST WATERS: WB AAM BATCH 23800

DILUTION MEDIA: WB

INOCULUM: WB

INOCULATED: WB

SAMPLED: HB

COUNTED: HB

TEST/CONTAINER VOLUME: ____

# REPLICATE FLASKS: ____

DILUTION RANGE: ____

NOTES: OTS Range Finding Tests

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE SC061989A-C CHEM. NUMBER OTN25600-602

SHAKER NUMBER ______ TEST MATERIAL ______

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	061989/JHB	2:30 pm	96	390-420
DAY 1	062089/JHB	2:00 pm.	96	390-420
DAY 2	062189/JHB	2:45 pm	96	390-420
day 3	062289/JHB	2:00 pm	96	390-410
DAY 4	062389/JHB	2:15 pm	96	390-410
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

TEST CODE: DC

TREATMENT:

SPECIES: Selenastrum

CHEM. #: OTN25600-602

SPIKE: AAM NEG. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	92	1	643	75	618	82	664	79
			2	711	77	709	78	699	80
			3	798	82	771	82	839	81
02	1 20	1159	1	1800	81	2253	78	2004	80
			2	2811	72	2663	73	2468	72
			3	3628	71	3811	71	3915	71
03	1200	2112	1	1167	65	1111	71	1081	71
			2	1362	72	1196	72	1241	72
			3	1341	67	1414	65	1345	69
04	1200	1016	1	4122	30	4221	30	4504	29
			2	5720	31	5761	30	5747	30
			3	5622	33	5763	32	6011	31
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: Zn POS. CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV	
01	1 20	92	1	493	51	479	46	452	43	1.48
			2	552	67	603	74	619	69	.74
			3	723	83	703	88	709	90	.38
02	1 20	1159	1	528	44	498	42	409	43	
			2	1709	65	1326	67	1709	65	
			3	2032	73	2054	74	1985	73	
03	1 20	2112	1	772	51	866	49	731	55	
			2	7931	60	7811	60	8151	59	
			3	7883	88	8502	82	7895	83	
04	1220	1216	1	1184	45	191	33	157	34	
			2	3314	27	3137	29	3144	28	
			3	3971	29	4155	27	4563	29	
			1							
			2							
			3							
			1							
			2							
			3							

TEST WATER: ______________________ TEST CODE: ______________________

TREATMENT: ______________________ SPECIES: Selenastrum

OTN25602

CHEM. #: N-octylamine SPIKE: 1000 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	192	1	430	37	880	37	718	36
			2						
			3						
02	20	159	1	983	41	385	39	457	37
			2						
			3						
03	20	212	1	484	38	424	31	421	36
			2						
			3						
04	20	1016	1	179	30	217	34	293	30
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 100 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	192	1	218	51	249	51	218	52
			2						
			3						
03	20	159	1	381	37	427	36	500	42
			2						
			3						
03	20	212	1	488	31	318	28	315	31
			2						
			3						
04	20		1	168	42	184	25	160	28
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: ____________________

TEST CODE: ____________________

TREATMENT: ____________________

SPECIES: Selenastrum

CHEM. #: 63 N-octylamine

SPIKE: 10 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	192	1	301	37	302	43	347	43
			2						
			3						
02	20	1519	1	353	26	325	27	303	28
			2						
			3						
03	20	2/12	1	436	29	412	29	378	20
			2						
			3						
04	20	1016	1	127	31	103	27	131	28
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 1 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	192	1	298	68	312	71	317	71
			2						
			3						
02	20	1519	1	322	51	370	39	357	40
			2						
			3						
03	20	2/12	1	215	40	238	43	225	47
			2						
			3						
04	20	1016	1	322	26	323	25	308	27
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: ____________

TREATMENT: ____________

TEST CODE: ____________

SPECIES: Selenastrum

CHEM. #: N-octylamine   SPIKE: .1 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	912	1	601	67	597	70	608	69
			2						
			3						
02	20	1519	1	21025	64	20219	65	19913	64
			2						
			3						
03	20	2112	1	19628	76	19592	75	19503	75
			2						
			3						
04	200	1106	1	13538	28	14712	28	14844	28
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: .01 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	912	1	487	69	498	70	519	68
			2						
			3						
02	20	1519	1	11681	66	11748	57	21818	59
			2						
			3						
03	20	2112	1	16719	74	16920	73	17147	73
			2						
			3						
04	200	1106	1	32119	28	33217	28	35115	28
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST CODE: SC 072489

TEST WATER: Nitrobenzene

CHEM NO. OTN 29600 (1000 mg/L stock)

DATE SAMPLED: —

DATE ELUTED: —

	high conc.	low conc.	
INITIAL pH:	7.4	7.4	
FINAL pH:	8.1	9.4	on 1310 h

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH:

INOCULUM:

Selenastrum ALGA

10⁴ CELLS/ML

.1620 MG/L DRY WT.

45 MCV

.4142 MLS INOCULUM

TEST PREPARATION:

TEST WATERS: SS AAM BATCH MEW 30800

DILUTION MEDIA: SS

INOCULUM: SS

INOCULATED: SS

SAMPLED: MS/WB

COUNTED: MS/WB

TEST/CONTAINER VOLUME: 50/125

REPLICATE FLASKS: 3

DILUTION RANGE:

NOTES: Procedure according to M.A. Bollman memo 013089 except #5

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE SG072489

CHEM. NUMBER ~~[illegible]~~ SS 0TN2960

SHAKER NUMBER 208

TEST MATERIAL Nitrobenzene

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	072489 SS	1310	100	410-430
DAY 1	072589 HB	14:00	100	410-440
DAY 2	072689 HB	14:10	100	410-430
day 3	072789 HB	13:15	100	360-410
DAY 4	072889 TMB	15:50	104	370-420
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

TEST WATER: Nitrobenzene

TEST CODE: SC072489

TREATMENT:

SPECIES: Selenastrum

CHEM. #: MEN308D

SPIKE: AAM NEGATIVE CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	126	170	1	11139	52	11133	52	11279	47
			2	11031	45	11710	50	11379	45
			3	17413	52	11667	51	11844	5[illegible]
02	120	167	1	5232	54	15036	55	5755	55
			2	14362	55	4[illegible]13	54	4594	54
			3	5327	55	531[illegible]	53	5448	57
03	150	145	1	20234	59	20925	57	20[illegible]06	51
			2	19723	57	18717	53	[illegible]15	51
			3	1897[illegible]	56	18931	54	17356	52
04	1200	1166	1	12293	44	12441	44	13966	43
			2	9583	47	11844	47	12097	46
			3	10461	47	11586	46	12289	46
			1						
			2						
			3						
			1						
			2						
			3						

CHEM. #: MCN1850

SPIKE: Zn++ POSITIVE CONTROL MCN1850

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	16	170	1	2814	78	1295	62	1360	53
			2	16[illegible]	[illegible]	572[illegible]	81	6[illegible]	75
			3	1658	51	631[illegible]	53	558	55
02	120	167	1	11053	51	11080	48	11029	50
			2	2[illegible]67	[illegible]	2153	72	21[illegible]05	47
			3	22277	66	2401	[illegible]	2471[illegible]	[illegible]6
03	150	145	1	11082	77	11[illegible]4	74	11[illegible]74	7[illegible]
			2	9341	53	10516	50	10[illegible]24	57
			3	10158	56	2573	54	10[illegible]7	57
04	1200	1166	1	1147	94	1280	71	1323	76
			2	6926	46	7088	45	7422	46
			3	7536	46	7628	45	7538	45
			1						
			2						
			3						
			1						
			2						
			3						

128ug/1 3
74ug/1 2
37ug/1 1

TEST WATER: Nitro benzene

TEST CODE: SC 072484

TREATMENT:

SPECIES: Sel

CHEM. #: OTN296D

SPIKE: 100 ppm

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	70	1	567	73	[illegible]	[illegible]	451	75
			2	547	60	[illegible]	65	547	71
			3	549	73	484	66	509	69
[illegible]	20	67	1	655	89	731	87	813	[illegible]
			2	509	82	453	83	463	[illegible]
			3	457	112	480	109	[illegible]	[illegible]
[illegible]	20	45	1	511	87	459	[illegible]	434	[illegible]
			2	380	93	[illegible]	[illegible]	365	[illegible]
			3	455	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	20	166	1	879	101	958	88	916	93
			2	595	91	596	92	604	103
			3	884	105	828	98	902	93
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 50 ppm

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	70	1	538	77	[illegible]	78	518	[illegible]
			2	711	78	[illegible]	77	741	[illegible]
			3	541	72	580	67	635	[illegible]
02	20	67	1	1195	76	1176	72	1260	[illegible]
			2	1401	74	1463	73	[illegible]	[illegible]
			3	1484	74	1458	71	1591	[illegible]
03	20	45	1	2235	62	[illegible]	52	[illegible]	[illegible]
			2	[illegible]	54	2518	66	[illegible]	[illegible]
			3	2979	67	2644	64	[illegible]	[illegible]
04	20	166	1	16337	54	17496	53	19122	54
			2	19036	52	17701	52	19016	51
			3	14333	53	14295	53	15490	52
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: Nitrobenzene

TEST CODE: SC072184

TREATMENT:

SPECIES: Sel

CHEM. #: OTN 2960

SPIKE: 25 ppm

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	70	1	278	50	10[illegible]	52	9[illegible]4	[illegible]
			2	1359	49	1323	47	1422	47
			3	1267	45	1478	45	1367	50
02	20	67	1	3689	51	3[illegible]59	53	3733	[illegible]
			2	4532	52	424[illegible]	51	5223	5[illegible]
			3	5135	56	5[illegible]	[illegible]6	5317	5[illegible]
03	[illegible]	45	1	8096	44	8956	47	8545	4[illegible]
			2	1876[illegible]	50	17568	47	[illegible]44	47
			3	21355	50	[illegible]	47	[illegible]	[illegible]7
04	200	166	1	7155	43	7842	43	7876	43
			2	8305	44	8032	42	8891	44
			3	8621	43	10043	43	9913	43
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 12.5 ppm

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	70	1	1178	48	1[illegible]	45	11[illegible]	5[illegible]
			2	15[illegible]	52	2154	45	1224	[illegible]
			3	1[illegible]28	5[illegible]	1344	[illegible]6	15[illegible]	5[illegible]
02	20	67	1	57[illegible]	51	5772	[illegible]2	6[illegible]	[illegible]
			2	4[illegible]1	53	486[illegible]	[illegible]2	4693	[illegible]
			3	4201	57	4421	61	450[illegible]	[illegible]
03	20	45	1	21705	53	19736	53	21[illegible]32	[illegible]
			2	18186	54	15684	53	17144	53
			3	20797	49	20563	[illegible]	21[illegible]7	4[illegible]
04	200	166	1	10237	53	9294	46	9982	47
			2	9261	46	10185	45	10671	44
			3	7481	46	10028	41	9997	41
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: Nitrobenzene

TEST CODE: ~~AF~~ SC 072484

TREATMENT:

SPECIES: Scl

CHEM. #: 0TN296n

SPIKE: 6.25 ppm

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	70	1	1102	56	1369	55	1310	56
			2	1509	51	9[illegible]4	49	1054	52
			3	1353	55	1452	53	1449	5[illegible]
02	20	67	1	4745	51	4939	51	4979	44
			2	4042	53	4061	52	4063	5[illegible]
			3	6067	55	6725	55	[illegible]	54
03	20	45	1	1[illegible]	49	17488	47	[illegible]	49
			2	22271	56	2444[illegible]	56	[illegible]	5[illegible]
			3	[illegible]	58	3[illegible]278	58	[illegible]	[illegible]
04	200	166	1	9820	50	10433	46	10190	49
			2	10346	43	9287	55	10519	52
			3	10943	44	12279	45	12403	45
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE:

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST CODE: OT071789

TEST WATER: NITROBENZENE

CHEM NO. OTN 77600

DATE SAMPLED: N/A

DATE ELUTED: N/A

	high conc.	low conc.
INITIAL pH:	7.5	7.5
FINAL pH:	7.3	9.77

ON @ 4:55 P

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 4

INOCULUM:

SELENASTRUM	ALGA
10^4	CELLS/ML
.1577	MG/L DRY WT.
43.9	MCV
.326	MLS INOCULUM

TEST PREPARATION:

TEST WATERS: MG AAM BATCH MEN26800

DILUTION MEDIA: MG

INOCULUM: MG

INOCULATED: MG

SAMPLED: MG

COUNTED: MG

TEST/CONTAINER VOLUME: 50/125

REPLICATE FLASKS: 1

DILUTION RANGE: 100 - .01 mg l

NOTES: O.T.S RANGE-FINDING TEST.

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE 0T071789

CHEM. NUMBER 8TN29600

SHAKER NUMBER 208

TEST MATERIAL NITROBENZENE

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	071789/ BOLLMAN	3:30	96	370-430
DAY 1	071889/ BOLLMAN	5:45	100	390-420
DAY 2	071989/ BOLLMAN	6:10	100	390-420
day 3	072089/ BOLLMAN	4:56	102	380-430
DAY 4	072189/ [illegible]	3 55	96	380-420
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

TEST WATER: NITRIBENZENE

TEST CODE: OTC71789

TREATMENT: N/A

SPECIES: Selenastrum

CHEM. #: MEH26400

SPIKE: AAM NEGATIVE CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	127	1	7898	56	8013	56	8165	52
			2	1494	49	1651	48	1517	58
			3	1218	54	1267	56	1195	53
02	20	377	1	6178	57	6105	56	6298	55
			2	6088	59	5913	57	6058	56
			3	5289	58	5211	56	5469	57
03	20	145	1	33359	58	34752	58	32185	60
			2	36697	60	37269	59	38248	57
			3	33600	59	33919	59	23507	60
04	200	102	1	13257	51	11682	52	12058	52
			2	16484	50	10719	50	12409	51
			3	11622	50	11949	49	10774	42
			1						
			2						
			3						
			1						
			2						
			3						

CHEM. #: MCN18500

SPIKE: Zn++ POSITIVE CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV	
01	20	127	1	298	74	218	86	274	75	128ug/1
			2	722	70	751	78	802	69	74ug/1
			3	734	48	733	41	775	42	37ug/1
02	24	377	1	1135	62	1121	64	1221	58	
			2	1396	61	1621	70	1594	63	
			3	7674	53	7624	52	7713	51	
03	20	145	1	3444	70	3350	62	3576	57	
			2	21782	60	22217	60	22628	60	
			3	45267	53	46647	52	47328	51	
04	200	102	1	848	51	1469	51	1128	51	
			2	7803	45	8222	45	830	[illegible]	
			3	11127	49	11921	49	11454	42	
			1							
			2							
			3							
			1							
			2							
			3							

TEST WATER: NitroBenzene

TEST CODE: 0T071789

TREATMENT: N/A

SPECIES: Selenastrum

CHEM. #: 0TN29600

SPIKE: 1000 µg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	1127	1	871	70	1012	72	1143	70
			2						
			3						
02	1 20	377	1	596	62	683	50	735	43
			2						
			3						
03	1 20	1145	1	972	48	1061	51	1214	54
			2						
			3						
04	1 20	1022	1	935	45	904	43	979	48
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 100 µg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	1127	1	378	61	431	66	505	55
			2						
			3						
02	1 20	377	1	426	77	450	76	1764	78
			2						
			3						
03	1 26	1145	1	885	84	904	75	892	91
			2						
			3						
04	1 20	1022	1	[illegible]	[illegible]	1078	108	1138	102
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: Nitrobenzene

TEST CODE: DT071789

TREATMENT: N/A

SPECIES: Selenastrum

CHEM. #: OTN39600

SPIKE: 10 mg/l

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	1127	1	11146	65	11431	75	11584	73
			2						
			3						
02	1 26	3177	1	3691	53	3853	52	4162	51
			2						
			3						
03	1 20	1415	1	36363	56	3093[illegible]	5[illegible]	[illegible]265	5[illegible]
			2						
			3						
04	200	1012	1	142[illegible]	46	14568	47	14904	47
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 1 mg/l

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	1127	1	872	51	887	50	949	[illegible]
			2						
			3						
02	1 26	3177	1	5901	55	6015	55	6234	54
			2						
			3						
03	1 26	1415	1	42411	58	43464	53	44309	5[illegible]
			2						
			3						
04	200	1012	1	13648	49	13620	49	1322[illegible]	44
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: NITROBENZENE

TEST CODE: DT071789

TREATMENT: N/A

SPECIES: Selenastrum

CHEM. #: DTN34600

SPIKE: 0.1 mg/l

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	127	1	7519	43	793	44	811	41
			2						
			3						
02	120	3177	1	16210	57	16908	57	8079	55
			2						
			3						
03	120	1145	1	38906	59	40390	59	42661	51
			2						
			3						
04	[illegible]	1012	1	13[illegible]9	47	13847	48	13999	47
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 0.01 mg/l

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	120	127	1	1014	45	998	49	1046	4[illegible]
			2						
			3						
02	120	3177	1	16001	58	16067	55	15709	54
			2						
			3						
03	120	1145	1	41689	63	42077	62	43056	63
			2						
			3						
04	1200	1[illegible]	1	14688	49	14869	49	15044	47
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST CODE: OT082889

TEST WATER: METHYL VINYL KETONE

CHEM NO. OTN34800

DN @ 13:15

DATE SAMPLED: N/A

DATE ELUTED: N/A

	high conc.	low conc.
INITIAL pH:	7.1	8.3
FINAL pH:	7.7	8.15

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 4

INOCULUM:

SELENASTRUM	ALGA
10^4	CELLS/ML
.1656	MG/L DRY WT.
46	MCV
.31	MLS INOCULUM

TEST PREPARATION:

TEST WATERS: MB AAM BATCH MEN

DILUTION MEDIA: MB

INOCULUM: MB

INOCULATED: MB

SAMPLED: MB/WB

COUNTED: MB/WB

TEST/CONTAINER VOLUME: 50/125

# REPLICATE FLASKS: 3

DILUTION RANGE: .5–.03 mg/L

NOTES: OT.S DEFINITIVE TEST. FOLLOWED SPECIAL O.T.S. TEST PROCEDURES.

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE BT 082889 CHEM. NUMBER OTN 34800

SHAKER NUMBER RM. 226 VENTED TEST MATERIAL METHYL VINYL KETONE

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	8/28/89 MB	13:15	100	360-390
DAY 1	8/29/89 MB	13:50	100	370-420
DAY 2	083089 / MB	16:00	100	370-410
day 3	083189 / MB	17:30	100	360-400
DAY 4	090189 / MB	18:00	100	380-420
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

TEST WATER: Methyl Vinyl Ketone

TEST CODE: OT082889

TREATMENT: N/A

SPECIES: Selenastrum

CHEM. #: MEN30800

SPIKE: AAM NEGATIVE CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
011	1 20	391	1	11632	60	11699	58	11687	57
			2	11259	62	11573	57	11581	51
			3	11866	54	11967	56	11779	48
012	1 20	1312	1	7920	65	8638	63	6898	63
			2	5991	61	4993	61	5872	61
			3	5970	64	7378	63	6176	61
013	1200	619	1	4810	64	5021	62	5246	65
			2	4542	65	4766	63	4943	62
			3	6556	63	6476	61	6531	75
014	1200	2185	1	12981	45	14090	44	15324	44
			2	15699	44	15027	43	14918	44
			3	20208	43	20833	43	22679	44
			1						
			2						
			3						
			1						
			2						
			3						

CHEM. #: MCN18500

SPIKE: Zn++ POSITIVE CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV	
011	1 20	391	1	605	85	558	74	573	79	128ug/1
			2	759	90	780	91	792	92	74ug/1
			3	1353	78	1355	68	1438	77	37ug/1
012	1 20	1312	1	2026	59	1845	54	2237	54	
			2	2991	88	3564	88	3350	84	
			3	8351	51	8425	52	8540	51	
013	1200	619	1	986	112	933	102	922	104	
			2	2406	80	2544	77	2647	77	
			3	4658	54	4594	58	4799	53	
014	1200	2185	1	804	73	1014	71	1010	71	
			2	7337	49	8144	47	8046	49	
			3	14029	43	14689	43	15175	44	
			1							
			2							
			3							
			1							
			2							
			3							

TEST WATER: Methyl Vinyl Ketone TEST CODE: OT082889

TREATMENT: N/A SPECIES: Sebastrum

CHEM. #: OTN34800 SPIKE: .5 mg/l

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	391	1	874	95	994	98	1010	94
			2	787	72	724	77	905	62
			3	903	73	871	74	907	75
02	20	132	1	605	45	565	45	699	46
			2	509	44	607	41	514	47
			3	651	45	533	46	610	44
03	20	169	1	878	108	965	104	779	86
			2	1529	86	1529	76	1932	88
			3	921	73	832	75	768	65
04	20	285	1	519	54	562	50	612	49
			2	487	54	524	52	562	48
			3	572	46	734	49	681	54
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: .75 mg/l

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	391	1	764	83	920	88	866	94
			2	731	64	773	75	877	85
			3	673	74	751	73	749	72
02	20	132	1	485	46	543	47	464	47
			2	552	44	487	45	543	45
			3	437	45	457	44	433	43
03	20	169	1	755	92	756	90	792	102
			2	689	80	712	75	731	81
			3	695	80	725	72	679	75
04	20	285	1	751	46	805	48	820	58
			2	555	44	573	51	627	48
			3	565	47	542	49	597	48
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: Methyl Vinyl Ketone

TEST CODE: OT082889

TREATMENT: N/A

SPECIES: Selenastrum

CHEM. #: OTH34800

SPIKE: .125 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	391	1	722	102	741	94	794	93
			2	639	76	695	74	712	76
			3	727	66	700	74	751	64
02	20	132	1	889	68	761	68	893	67
			2	839	64	787	61	864	61
			3	679	68	494	73	500	65
03	20	69	1	3078	71	2998	69	3155	69
			2	3952	65	3924	63	5280	63
			3	2046	62	2178	61	2169	60
04	20	285	1	34322	55	32292	55	35561	55
			2	40554	58	43973	57	42911	57
			3	23699	55	23213	54	24196	55
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: .0625 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	391	1	944	101	962	130	957	102
			2	939	102	979	92	930	96
			3	790	90	788	83	746	87
02	20	132	1	5977	48	6436	48	5128	47
			2	6998	50	6264	51	6936	51
			3	4393	58	4603	57	4504	55
03	200	69	1	4063	59	4254	62	4223	57
			2	5223	57	5592	54	5925	54
			3	4181	44	4443	50	4639	49
04	200	285	1	15653	41	16395	39	17076	43
			2	14857	39	16718	40	16979	40
			3	13321	42	14886	43	15185	43
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: Methyl Vinyl Ketone TEST CODE: OT082889

TREATMENT: N/A SPECIES: Selenastrum

CHEM. #: OTN34800 SPIKE: 0.0313 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	391	1	1267	70	1288	63	1343	63
			2	1873	67	2553	59	2018	57
			3	1632	62	1554	67	1541	60
02	20	132	1	5638	56	6881	56	6022	57
			2	7998	60	7512	60	8468	60
			3	8502	57	8242	57	8290	57
03	200	69	1	5299	65	5182	68	5149	64
			2	6700	61	6922	58	6810	59
			3	5794	61	5887	60	6079	58
04	200	285	1	16536	44	17845	44	19439	44
			2	19857	41	21035	42	20777	42
			3	18156	42	18813	42	18817	42
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE:

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST CODE: OT082189

TEST WATER: METHYL VINYL KETONE

CHEM NO. OTN34800

DATE SAMPLED: N/A

DATE ELUTED: N/A

ON @ 2:30

	high conc.	low conc.
INITIAL pH:	7.1	7.5
FINAL pH:	7.0	8.3

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 3

INOCULUM:

SELENASTRUM	ALGA
10^4	CELLS/ML
.1581	MG/L DRY WT.
44	MCV
.81	MLS INOCULUM

TEST PREPARATION:

TEST WATERS: MB AAM BATCH MEN30800

DILUTION MEDIA: MB

INOCULUM: MB

INOCULATED: MB

SAMPLED:

COUNTED:

TEST/CONTAINER VOLUME: 50/125

REPLICATE FLASKS: 1

DILUTION RANGE: 1000-.01 mg/L

NOTES: O.T.S. RANGE-FINDING TEST. FOLLOWED O.T.S. SPECIAL TEST PROCEDURES.

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE OT082189 CHEM. NUMBER OTN34800

SHAKER NUMBER RM. 226 VENTED TEST MATERIAL METHYL VINYL KETONE

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	WB / 8-21-89	14:30	100	360-390
DAY 1	HB / 8-22-89	14:00	104	360-390
DAY 2	HB / 8-23-89	14:15	104	360-390
day 3	WB / 8-24-89	15:15	104	360-390
DAY 4	HB / 8-25-89	15:00	104	360-390
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

CONTROLS FOR:

TEST WATER: METHYL VINYL KETONE

TEST CODE: OT082189

TREATMENT: N/A

SPECIES: Selenastrum

CHEM. #: MEN 30800

SPIKE: AAM NEGATIVE CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	92	1	2396	54	2394	53	2436	51
			2	2640	51	2644	53	2572	52
			3	2218	53	2253	54	2313	52
02	20	193	1	13830	55	15623	54	14723	54
			2	14896	58	14162	58	15589	58
			3	11779	59	13527	59	12453	59
03	200	212	1	6476	52	6371	52	7406	53
			2	7491	52	7240	52	8416	53
			3	5861	54	7387	54	7224	54
04	200	229	1	17968	42	17570	44	21160	42
			2	16092	43	19318	42	19117	42
			3	19091	42	17298	41	20985	41
			1						
			2						
			3						
			1						
			2						
			3						

CHEM. #: MCN 18500

SPIKE: Zn++ POSITIVE CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV	
01	20	92	1	932	49	961	49	984	50	128ug/1
			2	1371	78	1353	75	1413	77	74ug/1
			3	1964	59	1908	60	1890	63	37ug/1
02	20	193	1	1719	78	1378	75	1694	81	
			2	5388	74	6522	73	5927	73	
			3	12814	53	11628	54	13539	53	
03	200	212	1	462	51	589	54	619	57	
			2	3223	52	3783	54	3244	52	
			3	6603	49	5335	49	6892	49	
04	200	229	1	1034	75	1115	70	1008	68	
			2	7528	53	7211	54	7713	54	
			3	14738	47	16890	46	16454	47	
			1							
			2							
			3							
			1							
			2							
			3							

TEST WATER: Methyl Vinyl Ketone

TEST CODE: OT082189

TREATMENT: N/A

SPECIES: Selenastrum

CHEM. #: OTN34800

SPIKE: 1000 mg/l

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
011	1 20	192	1	822	42	848	43	867	41
			2						
			3						
012	1 20	1/193	1	839	45	959	40	934	43
			2						
			3						
013	1 26	21/12	1	788	39	961	43	918	39
			2						
			3						
014	1 20	2129	1	1258	51	948	43	1286	43
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 100 mg/l

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
011	1 20	192	1	778	44	754	42	856	41
			2						
			3						
012	1 20	1/193	1	911	39	888	37	992	41
			2						
			3						
013	1 26	21/12	1	780	39	765	41	814	38
			2						
			3						
014	1 26	2219	1	965	45	1094	43	992	40
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: METHYL VINYL KETONE

TEST CODE: OT082189

TREATMENT: N/A

SPECIES: [illegible]

CHEM. #: OTN34800

SPIKE: 10 mg/l

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	192	1	724	43	694	38	715	36
			2						
			3						
02	20	193	1	798	47	933	37	848	38
			2						
			3						
03	26	212	1	1264	37	1281	42	1261	39
			2						
			3						
04	20	229	1	979	49	846	42	1045	42
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 1 mg/l

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	26	192	1	823	44	788	45	794	43
			2						
			3						
02	20	193	1	761	40	803	42	879	37
			2						
			3						
03	26	212	1	704	41	775	43	816	44
			2						
			3						
04	20	239	1	975	41	1011	47	933	50
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: Methyl Vinyl Ketone

TEST CODE: OT082189

TREATMENT: N/A

SPECIES: [illegible]

CHEM. #: [redacted] OTN34800

SPIKE: 0.1 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	92	1	773	69	622	71	780	75
			2						
			3						
02	20	193	1	3418	62	3768	67	3604	62
			2						
			3						
03	20	212	1	26418	58	27772	59	28383	59
			2						
			3						
04	200	229	1	9551	50	10887	48	12932	48
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 0.01 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	92	1	2029	51	2003	49	1973	52
			2						
			3						
02	20	193	1	11008	55	10313	54	11699	56
			2						
			3						
03	200	212	1	5577	53	6331	54	6939	54
			2						
			3						
04	200	229	1	14186	44	17159	45	17079	45
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST CODE: OT091289A

TEST WATER: BUTYL MERCAPTAN

ON @ 10:30

CHEM NO. OTN37800

DATE SAMPLED: N/A

DATE ELUTED: N/A

	high conc.	low conc.
INITIAL pH:	6.5	6.4
FINAL pH:	9.2	9.6

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 4

INOCULUM:

SELENASTRUM	ALGA
10^4	CELLS/ML
.1613	MG/L DRY WT.
44.8	MCV
.544	MLS INOCULUM

TEST PREPARATION:

TEST WATERS: MB AAM BATCH 35800

DILUTION MEDIA: MB

INOCULUM: MB

INOCULATED: MB

SAMPLED:

COUNTED:

TEST/CONTAINER VOLUME: 50/125 ml

REPLICATE FLASKS: 1

DILUTION RANGE: 1000 - 0.01 mg/L

NOTES: O.T.S. SPECIAL PROCEDURES.

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE OT091289 A & B CHEM. NUMBER OTN37800-801

SHAKER NUMBER 226 VENTED. TEST MATERIAL METHYL ACRYLATE / BUTYL MERCAPTAN.

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	09/12/89 MB	10:20	100	370-400
DAY 1	09/13/89 MB	11:40	100	380-410
DAY 2	09/14/89 MB	13:30	100	380-410
day 3	09/15/89 HB	11:00	100	380-400
DAY 4	09/16/89 HB	10:30	100	380-400
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

TEST WATER: METHYL ACRYLATE & BUTYL MERCAPTAN. TEST CODE: OT091289A & B

TREATMENT: N/A SPECIES: Selenastrum

CHEM. #: MEN35800 SPIKE: AAM NEGATIVE CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	26 12	1	2298	40	2769	43	2459	43
			2	2156	40	2144	38	2295	37
			3	1492	42	1586	41	1556	41
02	1 20	16 15	1	12326	45	12320	44	12790	45
			2	13527	45	13777	46	14262	44
			3	12783	44	13297	44	13147	44
03	1 20	19 12	1	55910	48	55861	48	55803	47
			2	60324	47	59963	47	61378	47
			3	56691	48	57111	48	56876	48
04	1 200	17 12	1	18476	44	18401	43	20054	42
			2	20013	44	20746	44	19638	44
			3	18253	43	19422	43	20600	44
			1						
			2						
			3						
			1						
			2						
			3						

CHEM. #: MCN18500 SPIKE: Zn++ POSITIVE CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	26 12	1	696	69	737	65	695	66
			2	1408	79	1338	77	1372	71
			3	1712	40	1698	41	1739	39
02	1 20	16 15	1	2675	55	2846	52	3038	55
			2	5623	64	5680	62	5621	63
			3	13358	44	13503	44	13530	44
03	1 20	19 12	1	4731	68	4564	68	4289	68
			2	24750	48	24648	47	24801	48
			3	51831	47	53278	47	52980	47
04	1 200	17 12	1	706	71	831	72	882	71
			2	7569	46	7496	46	7662	47
			3	14516	50	14492	50	14371	50
			1						
			2						
			3						
			1						
			2						
			3						

148ug/1
74ug/1
37ug/1

TEST WATER: Butyl Mercaptan

TEST CODE: 0T091289A

TREATMENT: N/A

SPECIES: Selenastrum.

CHEM. #: 0TN 37800

SPIKE: 1000 mg/l

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	202	1	626	53	605	53	678	54
			2						
			3						
02	20	165	1	4365	51	5012	52	4386	51
			2						
			3						
03	20	192	1	15861	57	16082	57	16245	58
			2						
			3						
04	200	172	1	7912	59	7665	58	7825	58
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 100 mg/l

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	222	1	1856	41	1946	37	1937	36
			2						
			3						
02	20	165	1	10422	45	10625	44	10654	44
			2						
			3						
03	20	92	1	33679	48	37214	48	38441	48
			2						
			3						
04	200	172	1	15701	44	15479	44	15754	44
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: Butyl Mercaptan

TEST CODE: OT 091289A

TREATMENT: N/A

SPECIES: Selenastrum

CHEM. #: DTH 37800

SPIKE: 10 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	202	1	2558	40	2633	40	2551	39
			2						
			3						
02	20	65	1	14059	46	14302	46	14088	46
			2						
			3						
03	20	92	1	54481	47	55097	47	57044	47
			2						
			3						
04	200	172	1	19386	42	21271	44	21558	44
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 1 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	202	1	2131	41	2249	40	2466	41
			2						
			3						
02	20	165	1	13645	47	13957	47	14489	47
			2						
			3						
03	20	92	1	61831	49	65890	49	60949	49
			2						
			3						
04	200	172	1	16676	49	16750	49	17146	48
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: BUTYL MERCAPTAN

TEST CODE: OT091289A

TREATMENT: N/A

SPECIES: SELENASTRUM

CHEM. #: OTN37800

SPIKE: 0.1 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	202	1	2710	40	2855	39	2848	46
			2						
			3						
02	20	165	1	13690	46	13967	46	13980	46
			2						
			3						
03	20	92	1	54275	49	59875	48	66012	49
			2						
			3						
04	200	172	1	21728	40	21320	40	22699	39
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 0.01 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	202	1	2881	40	2954	40	2888	40
			2						
			3						
02	20	165	1	14465	45	15262	44	15690	45
			2						
			3						
03	20	92	1	52701	48	56539	49	58528	48
			2						
			3						
04	200	172	1	18700	45	19027	45	18141	44
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST CODE: OT09188GB

TEST WATER: Methyl Acrylate

CHEM NO. OTN 37801

DATE SAMPLED: N/A

DATE ELUTED: N/A

m 1530h

	high conc.	low conc.
INITIAL pH:	7.4	7.2
FINAL pH:	7.19	9.05

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 4

INOCULUM:

Selenastrum ALGA

10^4 CELLS/ML

.1433 MG/L DRY WT.

39.7 MCV

0.45 MLS INOCULUM

TEST PREPARATION:

TEST WATERS: SS AAM BATCH 35800

DILUTION MEDIA: SS

INOCULUM: SS

INOCULATED: SS

SAMPLED: ______

COUNTED: ______

TEST/CONTAINER VOLUME: 50/125 ml

REPLICATE FLASKS: 3

DILUTION RANGE: 100 - 6.25 mg/l

NOTES: OTS special procedures

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE OT091889A-B

CHEM. NUMBER ~~OTN378A-801~~ OTN37501

SHAKER NUMBER 226 vented

TEST MATERIAL ~~Methyl Acrylate~~/Butyl Mercaptan

DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
091889	1530	100	380-410
091989 TMB	1600	104	360-410
092089 TMB	1600	100	360-440
092189/HB	13:30	100	360-420
092289/HB	14:30	100	360-410

TEST WATER: Methyl Acrylate a Butyl Mercaptan TEST CODE: OT091889 A-B

TREATMENT: N/A SPECIES: Selenastrum

CHEM. #: MEN35800 SPIKE: AAM NEGATIVE CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	1 20	171	1	11345	143	11315	142	11316	140
			2	11884	143	11874	143	11943	142
			3	11693	141	11783	142	11695	140
02	1 20	69	1	16074	149	16162	150	16210	150
			2	17799	153	18048	152	17919	152
			3	18199	155	18317	155	18206	156
03	1 2000	301	1	14296	152	[illegible]	154	14193	150
			2	14495	152	14507	152	14672	153
			3	14351	151	14590	151	14851	153
04	[illegible]	[illegible]	1	[illegible]	[illegible]	[illegible]	148	[illegible]	147
			2	[illegible]	147	17494	148	[illegible]	[illegible]
			3	[illegible]	167	[illegible]	161	[illegible]	[illegible]
			1						
			2						
			3						
			1						
			2						
			3						

CHEM. #: MCN1850 SPIKE: Zn++ POSITIVE CONTROL

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV	
01	1 20	171	1	1544	171	1113	172	1686	187	148ug/1
			2	1750	181	1782	174	[illegible]	[illegible]	74ug/1
			3	11314	147	[illegible]	146	11341	156	37ug/1
02	1 20	63	1	11434	157	11483	162	11527	161	
			2	11872	161	11917	179	11968	181	
			3	17787	147	[illegible]	[illegible]	18302	147	
03	[illegible]	[illegible]	1	[illegible]	165	[illegible]	164	[illegible]	[illegible]	
			2	[illegible]	155	[illegible]	155	[illegible]	[illegible]	
			3	40769	151	44589	151	44057	151	
04	[illegible]	140	1	[illegible]	151	11373	155	11606	148	
			2	[illegible]	145	17634	145	18146	145	
			3	17440	143	18026	141	17844	142	
			1							
			2							
			3							
			1							
			2							
			3							

TEST WATER: Methyl Acrylate

TEST CODE: OT091889B

TREATMENT: N/A

SPECIES: Selenastrum

CHEM. #: OTN37801

SPIKE: 100 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	171	1	456	42	518	42	596	37
			2	466	41	526	35	545	37
			3	413	41	516	37	5[illegible]7	3[illegible]
02	20	168	1	567	43	471	46	527	42
			2	479	37	457	38	496	42
			3	507	41	524	44	611	43
03	20	301	1	674	45	683	48	734	48
			2	614	47	632	48	562	44
			3	597	49	611	47	604	43
04	20	180	1	790	41	645	39	605	39
			2	663	[illegible]	675	37	[illegible]	40
			3	579	44	[illegible]	[illegible]	613	35
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 50 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	191	1	518	38	561	34	594	[illegible]
			2	455	[illegible]	[illegible]	[illegible]	570	[illegible]
			3	524	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
02	20	65	1	573	82	546	45	529	[illegible]
			2	529	39	508	[illegible]	558	37
			3	[illegible]	[illegible]	593	34	633	42
03	20	301	1	561	47	617	44	627	43
			2	556	52	633	45	652	50
			3	681	51	707	50	715	52
04	20	[illegible]	1	910	44	930	41	881	43
			2	569	45	656	42	[illegible]	37
			3	1253	54	1269	[illegible]	1288	52
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: Methyl Acrylate

TEST CODE: OT091889B

TREATMENT: N/A

SPECIES: Selenastrum

CHEM. #: OTN37801

SPIKE: 25 mg/L

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	171	1	535	53	539	51	547	56
			2	593	60	573	62	578	63
			3	556	64	582	63	552	64
02	20	68	1	1727	51	1823	41	1948	49
			2	1777	54	1884	50	1832	49
			3	1819	50	1960	50	1984	54
03	20	301	1	4939	58	5110	58	5467	59
			2	7540	53	8132	53	7987	53
			3	10380	52	10989	51	10742	50
04	20	190	1	37647	56	42494	55	42[illegible]74	50
			2	65489	50	65028	51	66077	51
			3	[illegible]3111	49	44797	50	64800	50
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 12.5 mg/L

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	171	1	[illegible]73	[illegible]	72[illegible]	[illegible]	773	7[illegible]
			2	513	[illegible]	51[illegible]	[illegible]	[illegible]	7[illegible]
			3	5[illegible]	[illegible]	5[illegible]	[illegible]	15[illegible]0	[illegible]
	20	165	1	3165	70	3263	5[illegible]	3169	72
			2	2131	7[illegible]	2202	72	2138	7[illegible]
			3	2499	5[illegible]	2509	60	2562	5[illegible]
03	20	[illegible]	1	[illegible]9407	5[illegible]	30146	55	29803	55
			2	24206	5[illegible]	[illegible]3754	60	24800	58
			3	3[illegible]712	55	33637	53	22403	5[illegible]
04	200	190	1	14198	47	14960	47	14983	4[illegible]
			2	13[illegible]72	46	13611	46	13269	4[illegible]
			3	1[illegible]7[illegible]5	45	13052	45	13785	4[illegible]
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: Methyl Acrylate

TEST CODE: OT091889B

TREATMENT: N/A

SPECIES: Selenastrum

CHEM. #: OTN37801

SPIKE: 6.25 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	171	1	765	72	755	69	760	74
			2	786	71	768	66	842	63
			3	960	55	979	56	978	52
02	20	68	1	3494	59	3625	57	3797	60
			2	3658	51	3552	55	3597	52
			3	5069	47	5163	47	5310	47
03	200	3011	1	2407	53	3790	52	3161	50
			2	3137	51	3411	50	3231	47
			3	3773	46	[illegible]	47	4150	46
04	2000	1910	1	12091	49	12615	49	12716	49
			2	13613	43	12912	43	14879	43
			3	14276	42	14853	43	15131	43
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 3.13 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	171	1	[illegible]	46	[illegible]	[illegible]	[illegible]	[illegible]
			2	[illegible]	50	[illegible]	45	1005	56
			3	[illegible]	54	877	[illegible]	[illegible]	59
02	20	68	1	3597	56	3725	49	3964	45
			2	5021	51	5123	49	5234	49
			3	3548	46	3809	44	3818	42
[illegible]	[illegible]	[illegible]	1	3400	50	3715	50	3623	50
			2	[illegible]	50	[illegible]	51	4027	50
			3	3357	46	[illegible]	46	[illegible]	[illegible]
04	2000	1910	1	15005	43	16107	43	16484	43
			2	16005	42	[illegible]	42	17575	43
			3	16703	44	17321	44	[illegible]	43
			1						
			2						
			3						
			1						
			2						
			3						

TEST CODE: OT091289B

TEST WATER: METHYL ACRYLATE

CHEM NO. OTN37801

DATE SAMPLED: N/A

DATE ELUTED: N/A

	high conc.	low conc.
INITIAL pH:	6.4	6.3
FINAL pH:	7.7	9.3

STOCK CULTURE DATA:

MEDIA: AAM

DAYS GROWTH: 4

INOCULUM:

SELENASTRUM ALGA

10^4 CELLS/ML

.163 MG/L DRY WT.

44.8 MCV

.544 MLS INOCULUM

TEST PREPARATION:

TEST WATERS: MB AAM BATCH 35800

DILUTION MEDIA: MB

INOCULUM: MB

INOCULATED: MB

SAMPLED:

COUNTED:

TEST/CONTAINER VOLUME: 50/125 ml

REPLICATE FLASKS: 1

DILUTION RANGE: 1000-0.01 mg/l

NOTES: O.T.S. Special Procedures.

ALGAL TEST DAILY ENVIRONMENTAL PARAMETER MONITORING SHEET

TEST CODE OT091289 A & B CHEM. NUMBER OTN37800-801

SHAKER NUMBER 226 VENTED TEST MATERIAL METHYL ACRYLATE / BUTYL MERCAPTAN.

	DATE/ TECHNICIAN	TIME OF DAY OF MEASUREMENT	SHAKER SPEED (RPM)	LIGHT INTENSITY (FOOTCANDLES)
INITIAL	09/12/89 MB	10:20	100	370-400
DAY 1	09/13/89 MB	11:40	100	380-410
DAY 2	09/14/89 MB	13:30	100	380-410
day 3	09/15/89 JZ	11:00	100	380-400
DAY 4	09/16/89 JZ	10:30	100	380-400
DAY 5				
DAY 6				
DAY 7				
DAY 8				
DAY 9				
DAY 10				

TEST WATER: METHYL ACRYLATE

TEST CODE: OTD91289B

TREATMENT: N/A

SPECIES: SELENASTRUM

CHEM. #: OTN34801

SPIKE: 1000 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	202	1	684	45	730	45	778	42
			2						
			3						
02	20	165	1	700	38	735	38	778	40
			2						
			3						
03	20	192	1	1020	43	1034	45	1245	44
			2						
			3						
04	20	172	1	1246	58	1376	58	1322	57
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 100 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	202	1	803	40	516	42	821	45
			2						
			3						
02	20	65	1	828	40	868	41	832	42
			2						
			3						
03	20	92	1	1034	41	1019	41	967	45
			2						
			3						
04	20	172	1	907	53	972	53	925	54
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: Methyl Acrylate

TEST CODE: OT 091289B

TREATMENT: N/A

SPECIES: Selenastrum

CHEM. #: OTH37801

SPIKE: 10 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	202	1	992	63	1080	65	1099	59
			2						
			3						
02	20	165	1	7273	56	7101	56	7643	56
			2						
			3						
03	20	192	1	38742	54	45601	54	50013	55
			2						
			3						
04	200	172	1	16099	101	15983	101	15965	101
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 1 mg/L

DAY	DILUTION FACTOR	BACK-GROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	202	1	1416	46	1410	45	1523	43
			2						
			3						
02	20	165	1	12111	44	12130	44	12587	47
			2						
			3						
03	20	192	1	47812	46	53136	46	55537	46
			2						
			3						
04	200	172	1	17211	48	17062	47	16783	47
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

TEST WATER: Methyl Acrylate

TEST CODE: OT29289B

TREATMENT: N/A

SPECIES: Selenastrum

CHEM. #: OTN57801

SPIKE: 0.1 µg/L

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	202	1	1874	38	1877	37	1964	42
			2						
			3						
02	20	165	1	12557	45	12734	45	12844	45
			2						
			3						
03	20	92	1	51483	55	53720	56	55037	56
			2						
			3						
04	200	172	1	18757	44	18643	44	18722	43
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

SPIKE: 0,01 µg/L

DAY	DILUTION FACTOR	BACKGROUND	R	COUNTS	MCV	COUNTS	MCV	COUNTS	MCV
01	20	202	1	2203	37	2156	38	2144	37
			2						
			3						
02	20	165	1	13314	45	13442	44	13434	45
			2						
			3						
03	20	92	1	44254	58	51511	57	54187	59
			2						
			3	20208	42	19971	41	20579	41
04	200	172	1						
			2						
			3						
			1						
			2						
			3						
			1						
			2						
			3						

APPENDIX D

EDTA TEST RESULTS

FOLLOW UP SIDE-BY-SIDE ALGAL COMPARISON TEST OF NEGATIVE CONTROLS
WITH AND WITHOUT EDTA AND WITH AND WITHOUT TRACE CUPPER

This test was performed in six replicates each and three counts were taken of each flask.
(6 flasks each x 3 counts each = 18 counts per test)

ALL VALUES EXPRESSED IN CELLS / ML

	TEST1	TEST2	TEST3	TEST4
	AAM W/ EDTA W/ CU	AAM W/O EDTA W/ CU	AAM W/ EDTA W/O CU	AAM W/O EDTA W/O CU
1.	2380200	18680	2260400	21540
2.	2409400	19360	2299800	25120
3.	2538000	18400	2348600	19220
4.	3725400	20460	2101800	26320
5.	3916400	20540	2012800	26040
6.	4057200	21520	2142400	26720
7.	3455200	16940	2176400	19660
8.	3638200	18440	2266200	20000
9.	3776000	18440	2220200	19320
10.	3011800	21160	2914400	37680
11.	3260000	20840	2877000	36340
12.	3281800	20440	2913400	38360
13.	2456800	23120	2993000	23680
14.	2513400	23100	2977400	25940
15.	2543400	23280	3008000	26420
16.	2988200	13580	3638400	71900
17.	2983600	14160	3862000	76180
18.	2310000	14760	3865400	78860
	AVE. 3069167	AVE. 19284	AVE. 2715422	AVE. 34406

TEST DATE: 04-07-89